P E R T U S S I S V A C C I N E
S Y M P O S I U M

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VOLUME I

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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DIVISION OF BIOLOGICS STANDARDS

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PERTUSSIS VACCINE SYMPOSIUM

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Conference Room 4A
Building 31
National Institutes of Health
Bethesda, Maryland
Monday, October 21, 1963

The symposium was convened at 9:01 a.m., Dr. Roderick Murray presiding.

C O N T E N T S

(cont'd)

C O N T E N T S (continued)

C O N T E N T S (continued)

P R O C E E D I N G S

DR. MURRAY: Could I have your attention, please?

I must say it's a great pleasure to have everybody on board and seated almost to the minute before the meeting starts. I hope that this record for punctuality will continue throughout the rest of the meeting.

First of all, I want to welcome you to this meeting on pertussis vaccine. Its title is a "Pertussis Vaccine Symposium." I suppose one has to choose a name for any meeting.

Actually I should explain that this meeting was called as a result of a long-standing commitment with various producers and testing laboratories in order to air some of the potential problems that arise in the production and testing of pertussis vaccine.

We hope that in the course of this meeting there will be a full discussion of certain technical matters concerning potency testing of pertussis vaccine and of the toxicity testing, together with a good sprinkling of collateral subjects.

We had hoped that this meeting would stimulate additional investigative work and perhaps bring some light on some of the problems which have been encountered.

I would say that this meeting is definitely not designed to cover the entire field of pertussis. I regret

that there was misunderstanding among some people. We had inquiries from persons who wished to attend the meeting in the belief that this was a general meeting on pertussis. But because of the nature of the meeting itself, we felt that it would be more fruitful if it were limited to the number of people that you could get around a conference table.

As you can see, this has not been possible, and perhaps the meeting may be a little less spontaneous because of the larger number. We hope not, however.

Because of this interest, however, we have arranged to have a stenographic transcript of the proceedings.

No requirements were made for the submission of papers, so that we don't know how long each presentation will take. But I hope that you will confine yourself to the time set forth in the program.

And for those who do not have papers and are speaking from notes, the record will reflect what has been said.

I hope that you will keep this in mind when speaking and that you will enunciate clearly and that when discussions take place that these will be one person speaking at a time so that we can get a systematic record of what has been said.

Other than that, I just wish to extend our welcome to you and hope that the meeting will progress fruitfully.

A couple of remarks. We are here in Building 31. It is an office building of the NIH. And as far as luncheon facilities are concerned, these are in the cafeteria which is on the other side of that wall, so you don't have very far to go.

Having said that, then, I will turn the meeting over to Dr. Pittman.

DR. MARGARET PITTMAN: Thank you.

I have just a few more remarks. Dr. Murray has already said part of my speech.

First I want to welcome you here and thank you for your volunteering to participate in this meeting or to accept invitations to talk.

As you know, we sent out a request for suggestions. I have been able to incorporate most in the program. I did have to leave out two topics -- factors that influence potency testing and selection of strains of high antigenicity for vaccine production. No one volunteered.

I hope that these can be brought out in the discussion.

I want to add another topic: What should be the size of the booster dose?

As Dr. Murray says, we want this to be an informal meeting. There will be a recording. I might add that we do hope to publish a summary of the meeting in the Public Health Reports. And Dr. Feely and Mrs. Cox and Mrs. Gardner at my laboratory will be the rapporteurs.

Since the program is crowded, I do hope that you will keep in time. I brought my kitchen timer along for Dr. Kendrick to keep time on you, so there will be a little click.

Also besides lunch I think we'd better have a coffee break. The cafeteria closes at 10:30 so we must get out of here by 10:15 for the coffee break. And in the afternoon the cafeteria closes at 3:00, so we'll need to break about 2:45 if you want some coffee.

So now I think we're ready for our session, and I'd like to introduce to you Dr. Kendrick. She needs no introduction to people who work on pertussis.

Dr. Kendrick.

DR. KENDRICK: Thank you.

Well, we are starting it seems this morning with the idea that we have a vaccine that we think might work because we are starting out talking about the clinical responses.

And we're starting right in on these methods that are used or can be used for the evaluation of pertussis

vaccine.

We shall have first "Encephalopathy after pertussis immunization" by Dr. C. N. Christensen.

DR. C. N. CHRISTENSEN: Thank you, Dr. Kendrick.

Dr. Murray, Dr. Pittman, and Assembled Participants, you know that neurologic reactions have been recorded after various immunizing biologicals for a number of years, and as long as 30 years ago attention was drawn to the fact that these types of reactions could occur after pertussis immunization.

Perhaps knowledge of these occurrences was not really widespread until about 1948 when Byers and Mall published a collection of cases.

During that year a mouse toxicity test became part of the minimum requirements for the licensure of pertussis vaccine in this country, and shortly after that, at a round table discussion at the American Academy of Pediatrics, the hope was expressed that vaccines passing this test would have significantly less toxicity in children and that by their use encephalopathy might be eliminated or at least its incidence reduced.

Furthermore, in 1953 the test for pertussis vaccine potency was revised, and, in effect, a ceiling was put on maximum potency.

It had been thought that excessive potency

might possibly play a role, or it has been thought that excessive potency may play a role in reactions of this type, and it was hoped that by this measure once again the reactions might be lessened.

Well, we became interested in a facet of this about two years ago and began looking at the literature to see if there were any recent reports.

And as we looked at it, we found only four reports from 1955 onward, and actually if one looked at these it became obvious that the children upon whom the reports were made might well have received vaccines made under the old standards.

And so we decided that it would be worthwhile attempting to determine if reactions were still occurring.

It's easy to suppose that because there are no reports in the literature that reactions are not occurring. On the other hand, it seemed quite conceivable that because they were well known now people might not want to put them in the literature.

So to check on this point we decided to send out a little questionnaire, and we sent it to all of the professors of pediatrics in the country and to directors of children's hospitals, a total of 104 questionnaires.

At this point I want to say I'm grateful to all those that returned replies.

We asked if they had observed reactions in their institutions from 1955 to 1960, inclusive. We asked the age of the child, the sex of the child, what year the reaction occurred in, the time of its onset -- that is, in relationship to the injection, whether it was six hours or 12 hours after injection. We asked the preparation used and the source, the manufacturer.

We asked the order of the injection, and we also asked something about the outcome, whether the child had mental retardation or cerebral palsy or convulsions, what the situation was.

We did not ask for details of the clinical illness, and so from the reports that we received we are unable to verify any given diagnosis. Inasmuch as the people who received these questionnaires are well qualified individuals, being professors of pediatrics, we'll have to rest with their diagnosis as being correct I believe.

Well, we got back 75 reports, and 61 of them from 61 institutions showed there were no instances of neurologic reactions. But from 14 we had a reply that some type of reaction had occurred.

Now, two of them we can kind of discount as one was a child who had a single febrile convulsion shortly after the administration of a booster dose of an undetermined or undisclosed type of vaccine in 1956 and

was recovered completely and no further problems.

A second report concerned local paralysis in the leg which was a temporary thing, lasted less than ten days, presumably related to the injection technique -- too close to a nerve or something of that order.

Of interest is the fact that two of these injections were given in the vastus lateralis, the preferred site for avoiding this type of reaction or at least presumably the preferred site.

From the remaining 12 institutions we had reports of 21 children who apparently had some type of encephalopathy following pertussis immunization. Eleven were boys, and seven were girls, and the sex was not stated in three instances.

If I could have slide 1, now, please.

These are the ages of the children at the time the reaction occurred. Fifteen of them were under one year of age. For some reason we hadn't any two-year olds that had reactions. Then there was a sprinkling beyond that age group. The age is unknown in three children.

The higher incidence in the younger age groups I suspect reflects only the frequency with which immunizations are given in those age groups rather than a real tendency to affect the very young child.

(Slide 2)

In the next slide we can see that the reactions

have been sprinkled all through the years with no tremendous number in any one year, no concentration of any type.

(Slide 3)

The next slide shows that 17 of the reactions occurred after the use of triple antigen. DPT type unknown means, of course, fluid or possibly an adjuvant action, but it wasn't recorded in the questionnaire.

I think of interest is the fact that three of the reactions have occurred after quadruple vaccine.

The frequency after triple once again, like the age frequency, probably represents the frequency of use of preparations and not a tendency to occur after a particular type of preparation.

The manufacturers were identified for only three patients, and it was a different manufacturer each time, so we won't go into that any more than to state that is the situation.

(Slide 4)

The next slide shows that reactions were reported after each of the three injections which are customarily given for primary immunization as well as after booster injections.

This slide totals 23 rather than 21, the number of reactions, and the reason is that one child was recorded as having had a neurologic reaction after both his first and second injections and another child after both his second and third.

The details about the first child were not given in the report we received, but the second child was said to have febrile reaction with twitching following his first injection, and following the second one he had convulsions.

This, of course, would be against our usual recommendations, in that we would suggest, I think all of us, if neurologic manifestations are noted after one injection, further injections are probably not indicated.

The time of onset of the various reactions is that which is reported in the literature. As a matter of fact, 18 of these children had the reactions within 24 hours of the injection.

The most important part of this slide concerns the sequelae or the most important part of this presentation concerns the sequelae, an attempt to gain some insight into the severity of these reactions. If I could have the last slide, please. (Slide 5)

We asked specifically in the questionnaire whether the children had mental retardation or recurrent convulsions or if they had some other motor manifestations of cerebral palsy. And you can see that three children are said to have recovered completely. We do not know about four. But mental retardation was noted in a total of eight of the children, and recurrent convulsions have continued

to be manifested in 12 of the children.

Of some interest is that two of these children who have recurrent convulsions now had convulsions before they were given vaccine injections.

No deaths are reported in this group.

However, since a short summary of these findings was published in the American Journal of Diseases of Children a few months ago, I have had two inquiries come to me about further details we might have about these patients. And prompting both of these inquiries were children who had had some type of encephalopathy after immunization. In one of the children death had occurred.

So a fatal outcome is still possible.

It's obvious then that severe neurologic manifestations can still occur after immunization. We presume it's the pertussis component that is responsible here.

The mechanism of these reactions is still not clear. It doesn't seem to me that a sensitivity phenomenon is the real explanation inasmuch as it can occur after a first injection as easily as after a second or third or booster injection.

Furthermore, the onset is very short if this is a sensitivity type reaction, at least if it's delayed type reaction.

From the information that we have, we have no way of trying to arrive at an estimate of the incidence of these reactions. I can only say that two of the informants on these questionnaires volunteered the information in their institutions they thought reactions were occurring at about the same frequency as they have noted in the ten or 15 years prior to 1955.

In summary, by a questionnaire we attempted to determine whether encephalopathy was still occurring after pertussis immunization with vaccines which meet the current standards of potency and toxicity. It would appear that such reactions are still occurring inasmuch as we were able to collect 21 children from 12 institutions during the years 1955 to 1961. Fourteen of the children had mental retardation -- residual mental retardation, recurrent convulsions, cerebral palsy, or some combination of these defects.

Thank you very much.

(Applause)

DR. KENDRICK: Thank you, Dr. Christensen.

I'm sure that some of you will have things to contribute to this discussion when the time comes.

Dr. Murray I think has a little thing to say.

DR. MURRAY: I omitted to mention this at the outset, but in the interests of making a good record I hope

(Slide 1)

A G E

Age in Months	No. of Patients
1 - 3	5
4 - 6	7
7 - 12	3
25 - 36	1
Over 36	2
Unknown	3

(Slide 2)

YEAR OF REACTION

Year	No. of Patients
1955	3
1956	4
1957	3
1958	1
1959	2
1960	5
1961	2
Unk.	1

(Slide 3)

PREPARATIONS USED

DPT - Alum ppt.	12
DPT - $AlPO_4$	1
DPT - Type Unk.	4
Pertussis Vacc.	1
Quadruple Vacc.	3

(Slide 4)

INJECTION WITH WHICH

REACTION OCCURRED

First	8
Second	7
Third	3
Booster	2
Not Stated	3

(Slide 5)

SEQUELAE

M.R. - R.C.	5
M.R. - R.C. - C.P.	1
M.R. - C.P.	1
M.R.	1
R.C.	4
R.C. - C.P.	2
Complete Recovery	3
Not Stated	4

M.R. - Mental Retardation

R.C. - Recurrent Convulsions

C.P. - Cerebral Palsy

that the participants will be able to leave their slides with us in the order in which they presented them until tomorrow afternoon so that we can photograph them or reproduce them for the record.

We will do everything we can to expedite this, but we will certainly get the slides back to you tomorrow afternoon.

Another thing, and I hesitate to bring this up actually, and you will forgive me if I do mention it. It would be nice if after the lunch -- at least after the coffee break -- we could arrange matters so that auditors or nonparticipants would arrange themselves around the side chairs and that the actual participants in the conference whoare engaging in the presentation of papers and discussion could have the privilege of sitting at the table.

DR. KENDRICK: Thank you, Dr. Murray.

We will proceed immediately to our second paper and have a discussion on a standardized method for precision scoring of clinical reactions to vaccine.

Dr. Dale Barrett, of Detroit, will tell us how to do it.

DR. C. DALE BARRETT: Thank you, Dr. Kendrick.

It's certainly with a great sense of temerity that I present this work under such a title. Certainly the method is not perfect, and certainly it was designed as an

expediency originally to provide myself with a system for systematically collecting objective data on clinical reactions to vaccines.

The need for this became apparent in connection with the conduction of field trials of various vaccines over the past five years.

The vaccines under study have included various preparations of diphtheria, tetanus, pertussis, poliomyelitis, combined antigen, or, as we refer to it in this report, DPT Polio, as well as DPT combined antigen, polio vaccine alone -- that is, the inactivated -- and influenza vaccines.

The subjects have been children ranging in age from one month through five years. They were given their injections as, shall we say, outpatients actually. These were well children drawn from what normally would have been our case load in our well baby or child health clinics.

There were special investigational clinics set up for the purposes of the field trials, and these children were followed in their homes for signs of clinical reactions according to the procedure described in this report.

Earlier efforts to gather useful and reliable information by means of retrospective histories and spontaneous reporting by telephone or postcard from parents proved to be practically worthless for purposes of

evaluating the true incidence and severity of such reactions.

Now, this is not to say that retrospective histories and reporting by the parents do not have their place in conducting a field trial.

A method was sought, therefore, which would provide objective and reliable measurements and observations of all subjects under study within the first 24 hours after injection of the test vaccine and at extended intervals where indicated.

The manner in which the data are collected must be free of bias from all personnel associated with the giving of the injection, the observers, and from the parents of the children involved in the study.

A system of recording and scoring the observations needed to be devised which had the elements of simplicity, accuracy and reproducibility and yet provide sufficient sensitivity to reveal subtle but significant differences between the degrees of reactivity of the various vaccines under study.

Finally, the procedure must be reasonably economical to operate and require a minimum of professional personnel.

Now, I brought with me, in addition to the slides that will be shown shortly, 100 copies of the record form

which was designed and, incidentally, is still subject to improvement as future revisions become feasible. It's subject to improvement as far as I'm concerned in terms of format and design, but we are most receptive to any suggestions we might receive from this group in further revisions and improvement in the method and the form.

Along with the record there will be two supplemental mimeographed sheets which contain certain technical information interpreting the procedure and the record.

I brought my wares with me. For those who might have a special interest in the instruments used, we will have them for examination and comment at any time during this conference, but I won't take up that time now.

The method. Only especially trained nurses are used to make the home calls and collect and record the prescribed data. The preparation and training of these nurse observers is done by the medical director of the project, who sets up the standards under which they carry out their interviews and observations, which means that I go out in the field myself with the nurse that I recruit and train for this purpose and set up with this nurse the exact procedure for making the measurements and putting the questions, the interview questions, to the parent.

After this supervising the director will recruit other nurses as may be required and transmit the same procedure. And these personnel are spot-checked now and then to see that they are conforming to the intended procedure and standards.

Now, these nurse observers are not the same personnel used to assist in the clinic where the antigen injections are given. They have no knowledge as to which specific vaccine was administered. All they and the parents know is that the child is getting some shots designed to protect him against certain diseases.

The nurse observer makes her home calls the day following the antigen injection sometime between the hours of 8 a.m. to 8 p.m.

In some studies a second post-injection day call is made routinely. In others this may be done upon certain indications only.

I might say parenthetically here that the 48-hour call so-called -- it's actually anywhere in the period of the second day post-injection from 8 a.m. to 8 p.m. -- this we do routinely if we're working with a new vaccine or a new variation in the preparation of an old vaccine.

And to jump way ahead now, I would like to say that I have found that we can learn all we need to know

from the one-day for purposes of vaccine comparisons, but that doesn't mean we don't make additional visits to the home upon certain criteria if the child is exhibiting signs of reaction.

The criteria governing the indications for the prolonged followup home visits are stated in the material given you. The staff of nurses trained and employed for this purpose is kept as small as is practicable. This makes for a maximum efficiency and uniformity of technique in collecting the prescribed data.

Under certain conditions -- and these conditions are spelled out in the material given out -- a pediatrician is employed to make home calls for the purpose of assessing whether an underlying infection may also be present to account for the child's fever or other signs of illness.

Where the clinical findings are negative, the febrile state is automatically charged as reaction to the vaccine. But where there are concomitant signs of infection, the physician's clinical impression is noted on the record.

However, the vaccine under study is not necessarily absolved as a causative factor.

Subjects. Selection of subjects from each classification, such as age group and other demographic characteristics, was made at random so that each individual

has an equal probability of being assigned to any one of the various treatment schedules under study.

Instruments and techniques. An electronic thermometer is used for taking the temperature. The one that I selected happens to be an instrument known as the Tempo-Stat manufactured by the Stat Corporation of Needham, Massachusetts. It's calibrated in the Fahrenheit scale in two-tenths degree gradations.

It was found with this instrument that reasonable stability was reached at a 30-second time period and that it is of critical importance that the nurse make her reading within this 25- to 30-second period after the probe is placed in position.

Each electronic thermometer used was calibrated against a Bureau of Standards certified mercury laboratory thermometer prior to each day's usage.

A water bath adjusted to the temperature range 96 to 105 degrees was used as a medium for these calibrations.

The electronic thermometer had to agree with the reference thermometer within a tolerance of plus or minus 0.4 degrees.

It was decided to use the axillary temperature as the indicator of body temperature in preference to oral or rectal temperatures because of the economy of time gained

thereby and the elimination of the need for sterilizing the probe between subjects.

Axillary temperatures have proven in the hands of this investigator to be quite adequate and reliable means of measuring significant differences in body temperature reactions to various vaccines under study.

At each clinic visit the mother is advised to expect a call by a nurse on the following day. The purpose of the call is explained in such a way that the mother will be home, or if this is impossible she will at least have some responsible adult at the household so that the baby can be examined by the nurse and the history taken.

This has worked remarkably well, and it is because of this orientation, we feel, that we have had close to 100 per cent successful home visits.

The mother is also advised not to give the child any aspirin or other antipyretic medication for that matter until at least after the nurse makes her home visit the following day.

Measurements of skin temperature over the injection site. It also seemed to me that we would gain by having an objective evaluation, objective measurement of the heat at the injection site if a local inflammatory reaction were to occur. For this purpose we used two of our

Tempo-Stats, placing the probe of one over the injection site and the probe of the other on the opposite extremity approximating the same injection site area.

The nurse is able to manipulate both thermometers simultaneously and make a reading, and it's the difference between the two readings that is the crucial factor in assessing any heat at the local injection site.

To obviate the environmental temperature influence on the probe, three layers of polyfoam insulating material are wrapped around the probes, and the readings are made at 30 seconds just as for the axillary temperatures.

Unfortunately, the equipment we are using does not register below 95 degrees, and therefore we are very likely missing some significant differences. However, experience has shown that despite this limitation the procedure does reveal useful information at least in the more profound local reactions.

Another device utilized in these investigations is a template for measuring the approximate size of induration and erythema. This template is a sheet of hard plastic which has five holes corresponding to the diameters of 20, 30, 40, 60 and 80 millimeters. The nurse approximates the size of the palpable induration, if any, to the nearest circle size.

The same technique is used in measuring erythema.

The reaction evaluation report. I'm going to talk about the form that was distributed.

A form was designed to serve as a combination clinical record and data processing card. A sample has been given you. The McBee key sort card system was adopted for this purpose. This permits me as a clinical investigator to do my own punching -- that is, at my own staff level -- and my own sorting. I don't have to wait my turn for IBM processing which may take considerable time and doesn't give me workability of my own data that I'd like.

However, I believe that the questionnaire and the data columns or sections are designed so this would lend itself quite readily for conversion to the IBM punching.

A clerk completes the entries across the top of the section pertaining to the child's identification, date of injection, and so forth. Information pertaining to the identification of the vaccine though is omitted at this point so that the field nurse and medical director are not biased by having advance knowledge of these critical items.

Interview procedure. Upon arrival at the household under study, of the child under study, the field nurse adheres to the following procedure:

If at all possible, the child's mother is

interviewed. Sometimes it's impossible and we have to settle for the father and aunt or some other relative.

The following standardized question is asked: "How has your child been since your visit to the clinic?" That is the lead question, the first item of conversation, if you will, the nurse enters into upon reaching the mother, and she's ready to record right then and there with her chart.

The verbatim response of the informant is then recorded by the nurse.

This question was so structured as to preclude-- Oh, my goodness. Three more minutes. This question is structured so as to preclude any bias by the respondent in terms of the information recorded.

I'd like to show my slides now and we'll go through the rest of this in the next three or four minutes.

This is the electronic thermometer, about four inches across, and it's quite portable.

The next slide.

This is showing the calibration against the Bureau of Standards mercury thermometer.

Next shows the technique for placing a probe in the axilla.

Next, please.

The positioning, with the mother holding the

child's arm and the nurse making her reading at 30 seconds.

Next slide.

This is merely to show here that we standardize, of course, the site of injections as well as the rest of the procedure. This is our standard site.

Next, if we were to do deltoids this would be the site.

Next, the template that I spoke of for measuring induration and erythema is illustrated here.

Next, the nurse is making a home call. This is the greeting. And she's already putting her lead question.

Next, here she is recording. This is actually in one of the homes.

Next, the measurement of axillary temperature in a baby injected the preceding day.

Next, measurement of induration and erythema. There is actually some reaction here if we were to spend some time on that.

Here's the placement of the probes for the injection site and control site temperature taking.

Next, the polyfoam insulating material wrapped around and the reading now occurring at 30 seconds.

(Table 1a)

Now, some evidence of the method./ This is one lot of DPT-Polio antigen showing the reactivity after the first, second, third and fourth injections, the fourth

being a booster given some seven months after the series of the first three given a month apart.

The elements of this slide show that the axillary temperatures for the first three injections are essentially equivalent. There are some minor variations. But after the fourth, or booster injection, there is a definite rise in the higher ranges in terms of about 12 per cent showing reactions in the 101 up.

I want to say very quickly that we are watchful of very serious reactions all the way through here. I didn't run into any, and I don't consider this an alarming rise. It's just significant it did occur.

Next, illness complaints. (Table 1b) / This is in response to the history. I then grade that according to a pre-set system of scoring the complaint -- how was the baby since visit to clinic? And I don't have time now to go into this, but it roughly corresponds to the axillary temperature findings in this same vaccine.

Next, induration. (Table 1c) / Again there was essentially no difference between the first three injections, but the fourth injection does give an appreciable change.

This is a typographical error here. This should be "approximate diameter" instead of "circumference." Diameter is slightly greater with the booster. (Table has been corrected.)

Next. (Table 2) / This is a comparison of random lots of DPT

or triple DPT-Polio and of polio. The significant thing here is that DPT and DPT-Polio are essentially the same. The polio has a remarkably lower incidence of axillary temperature responses on the first day.

(Table 3a)

Next, the illness complaints./ This is a series of slides of a DPT, a quadruple, and a polio all made from the parent lots and we'll go through these to show the whole gamut of how the record can be useful in evaluating a vaccine.

This is three vaccines compared to one another. Again the polio is remarkably different reactions according to the complaints than the other vaccines. The axillary

(Table 3b)

temperatures show the same thing. / No axillary temperature responses beyond the normal range in the polio vaccine.

(Table 3c)

Next, indurations. / Again the same general pattern.

(Table 3d)

Next, erythema. / You might not think in Negro babies you could get much out of this, but our observers can.

(Table 3e)

Next,/ injection site temperatures also show a similar pattern.

(Table 4)

Next,/ here are two influenza vaccines, ten or 11 subjects each. This was a titration going from 1/2 to 1 to 1-1/2 to 2 on two different vaccines. One was treated a certain way; one was standard.

It is apparent that the Y vaccine is considerably -- less reactive /in fact, no reaction even as the dose increases -- whereas with the Z vaccine the axillary temperatures definitely came up.

I feel this is very good evidence that the method is sensitive, accurate, reliable and reproducible.

Thank you very much.

(Applause)

DR. KENDRICK: Thank you, Dr. Barrett. And this discussion will come later.

The next topic is evaluation of clinical reactions to vaccines, and Captain John Seal has graciously consented to substitute for Dr. J. A. Bell who could not be here.

Captain Seal.

CAPTAIN JOHN R. SEAL: Dr. Kendrick, Dr. Murray, Dr. Pittman, Ladies and Gentlemen: I'm glad Dr. Kendrick said I was substituting for Dr. Bell, as most of you would probably know my own experience with vaccines and reactions has been in military populations, and this is pretty well constrained to one age group.

Some of the important things though in evaluation are in connection with different age groups.

So being a substitute for Dr. Bell, I feel perfectly free to plagiarize some of his work, so I will really talk about Dr. Bell's work today.

Z Y X | DOSAGE 7 4T2 1 7 4U2 1 | MO. 7 4T2 1 7 4U2 1 AGE | 7 4T2 1 7 4U2 1 7 4 2 1 INJECTION NO. | PROJECT NUMBER 7 4T2 1 7 4U2 1 | CASE NUMBER 7 4TH2 1 7 4H2 1 7 4T2 1 7 4U2 1 | A-1374X

PREINJECTION TEMP. 7 4T2 1 7 4U2 1 — AXILLARY TEMP. - FIRST DAY 7 4T2 1 7 4U2 1 — AXILLARY TEMP. - SECOND DAY 7 4T2 1 7 4U2 1

CHILD'S NAME | DATE OF INJECTION | INJECTION NO | IM ☐ OTHER ☐ SC ☐ | PROTOCOL | PROJECT NO. | TREATMENT GP. | CASE NO.

ADDRESS | ZONE | DATE OF BIRTH | DOSE CC. | RT. BUTTOCK ☐ ① LT. BUTTOCK ☐ ② RT. DELTOID ☐ ③ LT. DELTOID ☐ ④ ○ | SEE REVERSE SIDE FOR ADDITIONAL REMARKS ☐ ☐

PHONE | AGE AT TIME OF INJECTION ☐ MOS. ☐ YRS. | WEIGHT LBS. | PREINJECTION AXILLARY TEMPERATURE _____ •

WHITE ⑤ | NEGRO ⑥ | OTHER (SPECIFY) ⑦ | MALE ⑧ | FEMALE ⑨

FIRST POSTINJECTION DAY

DATE | TIME | SIGNATURE | ☐ DOCTOR ☐ MOTHER ☐ NURSE

1. HISTORY A. HOW HAS CHILD BEEN SINCE VISIT TO CLINIC?

B. HAS CHILD BEEN FEVERISH? NO ☐ ⑩ YES ☐ ⑪

C. HAS CHILD BEEN UNUSUALLY FUSSY? NO ☐ ⑫ YES ☐ ⑬

D. INFORMANT MOTHER ☐ ⑭ OTHER ☐ ⑮ (SPECIFY) _____

MEDICAL DIRECTOR'S EVALUATION (SEVERITY SCORE)

GRADE 0 ☐ ⑯ 1 ☐ ⑰ 2 ☐ ⑱ 3 ☐ ⑲ 4 ☐ ⑳ N.S. ☐ ㉑

2. INDURATION AT INJECTION SITE *

GRADE	0 ㉒	1 ㉓	2 ㉔	3 ㉕	4 ㉖
DIAMETER	NONE	0-20 MM	20 MM / 30 MM / 40 MM	60 MM / 80 MM	OVER 80 MM (SPECIFY SIZE)
CIRCLE SIZE	☐	☐	NO. 1 ☐ NO. 2 ☐ NO. 3 ☐	NO. 4 ☐ NO. 5 ☐	☐

3. REDNESS AT INJECTION SITE

GRADE	0 ㉗	1 ㉘	2 ㉙	3 ㉚	4 ㉛
DIAMETER	NONE	0-20 MM	20 MM / 30 MM / 40 MM	60 MM / 80 MM	OVER 80 MM (SPECIFY SIZE)
CIRCLE SIZE	☐	☐	NO. 1 ☐ NO. 2 ☐ NO. 3 ☐	NO. 4 ☐ NO. 5 ☐	☐

4. AXILLARY TEMPERATURE

GRADE	0 ㉜	1 ㉝	2 ㉞	3 ㉟	4 ㊱
RANGE	BELOW 99.0	99.0-99.9	100.0-100.9	101.0-102.9	103.0 & OVER
READING	_____ •	_____ •	_____ •	_____ •	_____ •

5. SKIN TEMPERATURE

	READING	DIFFERENTIAL 0 ㊲	1 ㊳	2 ㊴	3 ㊵	4 ㊶
INJECTION SITE	_____ •					
CONTROL SITE	_____ •	LESS THAN 1.0 ☐	1.0 1.9 ☐	2.0-2.9 ☐	3.0-3.9 ☐	4.0 & OVER ☐
DIFFERENCE	_____ •					

SECOND POSTINJECTION DAY

DATE | TIME | SIGNATURE | ☐ DOCTOR ☐ MOTHER ☐ NURSE

1. HISTORY A. HOW HAS CHILD BEEN IN PAST 24 HOURS?

B. HAS CHILD BEEN FEVERISH? NO ☐ ㊷ YES ☐ ㊸

C. HAS CHILD BEEN UNUSUALLY FUSSY? NO ☐ ㊹ YES ☐ ㊺

D. INFORMANT MOTHER ☐ ㊻ OTHER ☐ ㊼ (SPECIFY) _____

MEDICAL DIRECTOR'S EVALUATION (SEVERITY SCORE)

GRADE 0 ☐ ㊽ 1 ☐ ㊾ 2 ☐ ㊿ 3 ☐ (51) 4 ☐ (52) N.S. ☐ (53)

2. INDURATION AT INJECTION SITE *

GRADE	0 (54)	1 (55)	2 (56)	3 (57)	4 (58)
DIAMETER	NONE	0-20 MM	20 MM / 30 MM / 40 MM	60 MM / 80 MM	OVER 80 MM (SPECIFY SIZE)
CIRCLE SIZE	☐	☐	NO. 1 ☐ NO. 2 ☐ NO. 3 ☐	NO. 4 ☐ NO. 5 ☐	☐

3. REDNESS AT INJECTION SITE

GRADE	0 (59)	1 (60)	2 (61)	3 (62)	4 (63)
DIAMETER	NONE	0-20 MM	20 MM / 30 MM / 40 MM	60 MM / 80 MM	OVER 80 MM (SPECIFY SIZE)
CIRCLE SIZE	☐	☐	NO. 1 ☐ NO. 2 ☐ NO. 3 ☐	NO. 4 ☐ NO. 5 ☐	☐

4. AXILLARY TEMPERATURE

GRADE	0 (64)	1 (65)	2 (66)	3 (67)	4 (68)
RANGE	BELOW 99.0	99.0-99.9	100.0-100.9	101.0-102.9	103.0 & OVER
READING	_____ •	_____ •	_____ •	_____ •	_____ •

5. SKIN TEMPERATURE

	READING	DIFFERENTIAL 0 (69)	1 (70)	2 (71)	3 (72)	4 (73)
INJECTION SITE	_____ •					
CONTROL SITE	_____ •	LESS THAN 1.0 ☐	1.0 1.9 ☐	2.0-2.9 ☐	3.0-3.9 ☐	4.0 & OVER ☐
DIFFERENCE	_____ •					

*MEASURE PALPABLE INDURATION USING SCALE PROVIDED AND CHECK BOX CORRESPONDING TO NEAREST CIRCLE SIZE

REACTION EVALUATION REPORT REV. 7-1-72

C. D. BARRETT JR. M.D. DIRECTOR, MATERNAL & CHILD HEALTH DETROIT DEPT. OF HEALTH

CRITERIA FOR RECORDING AND SCORING COMPLAINTS

Mothers were asked by the field nurse "How has your baby been since your visit to the clinic?" The verbatim response was recorded by the nurse and evaluated by the medical director. The severity of the complaint, if any, was scored as follows:

Grade 0 - No complaints

Grade 1 - Mild or borderline complaints; for example: Child was "fussy" or "fretful" and/or had "slight fever." This type of complaint, in the opinion of the evaluator, appeared to be rather trivial and inconsequential.

Grade 2 - Moderately severe complaints; for example: Child "very hot", "feverish and irritable", "feverish all night", "cried more than usual during night". There seemed to be no question, in the opinion of the evaluator, that these complaints were definitive and valid.

Grade 3 - Very severe complaints; for example: Child sufficiently ill as to motivate mother to take him to a physician or hospital for treatment, occurrence of repeated vomiting, a convulsive episode, urticaria, etc.

Grade 4 - Permanent sequelae or death attributable to antigen injection.

10-15-63

REVISED CRITERIA FOR SCORING REDNESS AND INDURATION AT INJECTION SITE

CODE	DIAMETER
1	0
2	Equal to or less than 2 cm
3	Equal to or less than 3 cm
4	Equal to or less than 4 cm
5	Equal to or less than 6 cm
6	Equal to or less than 8 cm
7	Greater than 8 cm

CRITERIA FOR EXTENDED DAILY HOME VISITS BY NURSE

1. INDURATION at injection site of 30 mm or more.
2. REDNESS at injection site of 30 mm or more.
3. AXILLARY TEMPERATURE of 100.0°F or higher.
4. INJECTION SITE/ CONTROL SITE SKIN TEMPERATURE differential of more than 1.0°F.
5. Any sick or suspiciously ill child, who in the judgment of the nurse, regardless of the above findings, should be kept under prolonged observation.
6. Any sick child who in the judgment of the pediatric consultant to the project should be further followed by the nurse.

CRITERIA FOR HOME VISIT BY PEDIATRIC CONSULTANT

1. Mandatory for any child with an axillary temperature of 101.0°F or more.
2. Mandatory for any child showing suspicious signs of an urticarial eruption or other "allergic" manifestations.
3. Optional in the judgment of the nurse wherever she feels a medical evaluation of an ill child is warranted. (This perogative is to be used with great discretion by the nurse-observers so as to not abuse the services of the project physician in making unnecessary house calls.)

NOTE: In all of the above instances, if the nurse finds a critically ill child, she is instructed to consider this as a medical emergency and to advise the parent (or parent-substitute) to take the child immediately to the hospital.

Table 1a. AXILLARY TEMPERATURE 1-day following DPT-Polio antigen*injections

njection Number	Total Cases	Temperature range <99.0°	99.0-100.9°	101.0-102.9°	103.0° & Over
		%	%	%	%
1	97	44.3	53.6	2.1	0.0
2	92	32.6	65.2	2.2	0.0
3	52	36.5	61.5	1.9	0.0
4**	84	27.4	60.7	10.7	1.2***

Project 16-Lot 2013 **Booster ***Highest: 103.0°

Table 1b. ILLNESS COMPLAINTS

jection Number	Total Cases	Severity Score Grade 0	Grade 1	Grade 2	Grade 3
		%	%	%	%
1	98	17.4	62.2	19.4	1.0
2	92	18.5	32.8	48.7	0.0
3	49	14.3	40.8	44.5	0.0
4**	80	13.7	33.7	51.2	1.4

roject 16-Lot 2013 **Booster

Table 1c. INDURATION AT INJECTION SITE

Injection Number	Total Cases	Approximate diameter 0-10 mm	15-25 mm	30-50 mm	60-80 mm
		%	%	%	%
1	98	94.9	3.1	2.0	0.0
2	89	85.5	12.3	2.2	0.0
3	52	80.7	13.5	3.9	1.9
4**	85	67.0	8.2	23.6	1.2

*Project 16-Lot 2013 **Booster

Table 2 AXILLARY TEMPERATURE 1-day following FIRST injection with DPT, DPT-Polio, and Polio antigens

Antigen	Total Cases	Temperature range <99.0°	99.0-100.9°	101.0-102.9°	103.0° & Over
		%	%	%	%
DPT*	58	55.2	39.6	5.2	0.0
DPT-Polio**	86	53.6	42.9	3.5	0.0
Polio***	22	91.0	9.0	0.0	0.0

*Project 18 **Project 16-Lot 2012 ***Project 18

Table 3a. ILLNESS COMPLAINTS 1-day following BOOSTER injection with DPT, DPT-Polio and Polio antigens

Antigen	Total Cases	Severity Score Grade 0	Grade 1	Grade 2
		%	%	%
DPT*	49	10.0	45.0	45.0
DPT-Polio**	46	8.7	43.5	47.8
Polio***	48	79.2	16.6	4.2

*Dose: 1/2 cc., Lot x-094137 Site: Left Deltoid **Dose: 1 cc., Lot X-9213 Age range: 1-5 years ***Dose: 1 cc., Lot X-9306 Project 22

Table 3b. AXILLARY TEMPERATURE

Antigen	Total Cases	Temperature range <99.0°	99.0-100.9°	101.0-102.9°
		%	%	%
DPT	51	37.2	43.2	19.6
DPT-Polio	50	30.0	60.0	10.0
Polio	49	100.0	0.0	0.0

Project 22

Table 3c. INDURATION AT INJECTION SITE

Antigen	Total Cases	Approximate diameter 0-10 mm	15-25 mm	30-50 mm
		%	%	%
DPT	51	72.6	11.7	15.7
DPT-Polio	50	66.0	16.0	18.0
Polio	49	95.2	2.4	2.4

Project 22

Table 3d. ERYTHEMA AT INJECTION SITE

Antigen	Total Cases	Approximate diameter 0-10 mm	15-25 mm	30-50 mm
		%	%	%
DPT	51	78.4	15.7	5.9
DPT-Polio	50	88.0	8.0	4.0
Polio	49	97.6	2.4	0.0

Project 22

Table 3e. INJECTION SITE TEMPERATURE

Antigen	Total Cases	Severity Score Grade 0	Grade 1	Grade 2
		%	%	%
DPT	51	74.5	19.6	5.9
DPT-Polio	50	72.0	20.0	8.0
Polio	49	93.9	6.1	0.0

Project 22

Table 4 T OLERANCE TITRATION OF TWO INFLUENZA VACCINES

BASED UPON AXILLARY TEMPERATURE REACTION 1-DAY FOLLOWING INJECTION

Axillary Temperature	Dose 0.5 cc Vaccine Y	Dose 0.5 cc Vaccine Z	Dose 1.0 cc Vaccine Y	Dose 1.0 cc Vaccine Z	Dose 1.5 cc Vaccine Y	Dose 1.5 cc Vaccine Z	Dose 2.0 cc Vaccine Y	Dose 2.0 cc Vaccine Z
96.8							*	
97.0	*							
.2	**	*	*		*			
.4	*		*					
.6	*		*		*			
.8	*m				***			
98.0	**	*	**		***m		****m	
.2	*		*m		*		**	
.4			*	*	*	*	*	
.6	*	*	**	*	*			
.8			**	**	*	*	**	
99.0		*		*				
.2		**m				**		
.4		*		*m		*		
.6		*		*				
.8		*				*m		
100.0				*		*		
.2				*		*		
.4				**		*		
.6		*				*		
.8								
101.0								
.2		*						
.4	*					*		

m = median

* = one observation

Project 20

CDB:js

One of the things we have to think about really is why do we want accurate evaluation of reactions to vaccines?

The first reason I would say important to both a manufacturer and a physician is how acceptable will this vaccine be to his clientele.

If the reaction rate is too high, it will not be acceptable unless the disease which it is designed to prevent is a very, very serious disease and very likely to occur in the population.

The second main reason is that we must know whether the incapacitation produced by a vaccine exceeds that of the illness which it is designed to prevent.

This is also a problem with influenza vaccine, and I'm sure many of the opinions which existed prior to 1957 were thatinfluenza vaccine is not worthwhile for this very reason.

The third is that in attempts to arrive at a satisfactory dose response adjustment of vaccine we must know what doses produce which reactions with a high degree of accuracy.

Our problems in clinical evaluation are very much complicated by psychogenic reactions. These can originate from the attitudes and beliefs of the individual who administers the vaccine, from the attitudes and beliefs

of the recipient of the vaccine, from publicity -- that is, which may influence both of these -- and from the prior experience of the recipient.

They are further complicated by the fact that in every population there are a number of minor illnesses which occur routinely, and these may or may not occur in relation to the administration of the vaccine -- the common cold, other illness particularly in childhood populations.

Then there are the problems of variation from lot to lot of vaccines. So in order to get a really accurate evaluation there is need for the double blind technique which Dr. Barrett has mentioned, in which neither the recipient nor the evaluator is aware of the identity of the vaccine received until after the results are in and the analysis is complete.

There is a need for inclusion of a control particularly at the beginning of a vaccine's evaluation in order to measure some of the psychogenic factors and the occurrence of incidental illnesses.

I think the best study that I know of which illustrates some of these problems is that which Dr. Bell of the National Institutes of Health, Dr. Seltzer and Dr. Sartwell of Johns Hopkins conducted with influenza vaccine back in 1957, and this is published in the American Journal of Hygiene, Volume 75, in January 1962.

The vaccine that they set out to evaluate was a monovalent Asian strain influenza vaccine containing 500 CCA per milliliter, and this was provided by Dr. Murray's laboratory here at the National Institutes of Health.

A control was made up by a manufacturer which contains sterile saline and the same amounts of formalin and merthiolate which were included in the actual vaccine.

With full knowledge that children tend to react to influenza vaccines with fairly severe reactions, there was a dose adjustment, ages 1 to 5 receiving one-tenth of a milliliter, 50 CCA, ages 6 to 11 one-quarter of a milliliter, 125 CCA, and then all over 12 receiving a half a milliliter, 250 CCA.

The total number were 1,617 vaccinated, 1,563 controls. And these were in Montgomery County families that had participated in prior studies conducted by the National Institutes of Health.

The system of selection of these people for vaccine and for control injection was totally randomized.

Could I have the first slide, please? (Slide 1)

These slides are from the paper by Seltzer, Sartwell and Bell.

I think this slide I will use illustrates two major points, one of which is that fever in the vaccinated group was considerably above that in the control group,

although 1.3 per cent of the control group did develop fevers within 24 hours after the vaccine.

Then as you move out into the next 48-hour period after the first 24-hour observation there are still fevers in both groups but essentially the same incidence.

So practically all the fevers due to the vaccine were within the first 24 hours.

If it had been a single observation at three days, much of this would have been diluted out.

These systemic symptoms, headaches, malaise, nausea, chills, muscle aches and vomiting, were also significantly higher in the vaccinated group during the first 24 hours than in the control group.

Again as you move out into the next 48-hour period this difference disappears.

The number of other symptoms, coryza, sore throat and what not, occurred with equal frequency in both groups at all times.

Could I have the next slide, please? (Slide 2)

This slide actually summarizes the constitutional reactions which were significant in the previous slide. Here again is the dose range by age group, and you see this has been adjusted to minimize reactions in the children.

There is a clear difference in the occurrence of constitutional reactions in the vaccinees versus controls.

And then in this column -- and I don't have the time to explain it -- is the estimated frequency actually due to the vaccine. This was a rather complicated procedure. You have to assume that the individual may have a reaction due to something else, psyche or something of that sort, plus the vaccine reaction, to calculate this. But this is the general frequency.

I think you see that it has been adjusted so the incidence -- by adjustment of those -- so the incidence is relatively uniform.

Next slide, please. (Slide 3)

This is the frequency of local reactions by age in vaccinated and control groups. These are the numbers of reactions that occurred, that had local reactions, in the vaccinated group versus the control group, and the per cent, again a clear difference.

Next slide. (Slide 4)

Dr. Bell and his colleagues were concerned with incapacitation and defined it as absence from work or school for eight hours, a day away from normal duties, or in the case of a housewife an inability actually to carry on her household duties during a one-day period.

Since children under the age of five it would be hard to apply such criteria to, this applies only to those who were six years of age or higher and in school. And you

see again a clear difference in the amount of incapacitation between the vaccinated and the control.

But I do want to make the point that in the control group two per cent of the total of 1,500-odd people had an incapacitating illness of some kind due to either psychogenic reaction or some intercurrent illness within the two or three days following receipt of a control material.

I think you can see that there is a considerable dropoff as you move into adult populations here also.

Next slide. (Slide 5)

This is the last slide, which is the duration of incapacitation following inoculation for those incapacitated. On the number of days of incapacitation in the vaccinated group, you can see practically all of them were one day, 81 per cent, with only 3 per cent being three or more days of incapacitation.

In contrast, in the control group, 22 per cent were three or more days' duration. And this I think speaks highly for the intercurrent illness problem in a control population.

I think that's all the slides.

So in essence, to evaluate this particular influenza vaccine and to allay many of the fears in the country about going into mass programs, a control study of this sort did indicate that there were illnesses, reactions

to the vaccine, and that these in general were mild, occurred in a significant number of people, but only about 4 per cent of the total recipients had an incapacitating illness, and this was usually one day or less.

The influenza, on the other hand, particularly in the 1957 situation, offered a much bigger threat to the country as a whole in terms of incapacitation, not to mention death in elderly and so forth.

So I think the logical conclusion of this study, and well fortified with good data, is that it is perfectly safe to use this vaccine in the majority of the civilian population as long as the dose in children was kept adjusted.

Thank you.

(Applause)

DR. KENDRICK: Thank you, Captain Seal.

We thank all of the people who have kept within their time limits, and so you are going to get a coffee break. Otherwise you wouldn't.

So I think we can have a coffee break now for 15 minutes, and then we will reassemble after 15 minutes.

(Whereupon, a recess was taken.)

DR. PITTMAN: You did take 20 minutes, but there was a long line in the cafeteria. We got there and met some of the people of the building having coffee. So I'm sorry you had a little long line, but we were ahead of

5

Duration of incapacitation following inoculation, for those incapacitated

Duration (days)	Vaccinated group		Control group	
	No.	Per cent	No.	Per cent
1	69	81	18	67
2	13	15	3	11
3	2	2	4	15
4 or more	1	1	2	7
Totals	85	100	27	100

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38 A

2

Constitutional symptoms occurring in study population during first 72 hours following inoculation, by age*

Age group (years)	Dosage, CCA units	Vaccinees		Controls		Estimated true frequency attributable to vaccine (per cent)
		No.	Per cent	No.	Per cent	
1–5	50	37	15.5	18	8.1	8.1
6–11	125	62	15.2	24	4.9	10.8
12–19	250	24	14.4	11	6.6	8.4
20–39	250	60	14.5	34	7.6	7.5
40 and over	250	33	12.8	18	7.5	5.7
All ages		236	14.6	105	6.7	8.5

* Fever, headache, malaise, nausea, vomiting, chills or muscle aching.

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Frequency of incapacitating illness following inoculation (Ages 1–6 omitted)

Age group (years)	Vaccinees		Controls		Estimated true frequency
	No.	Per cent	No.	Per cent	(per cent)
6–11	50	9.3	12	2.5	7.0
12–19	15	9.0	3	1.8	7.3
20–39	15	3.6	8	1.8	1.8
40 and over	5	1.9	4	1.7	0.2
All ages	85	6.2	27	2.0	4.3

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Frqeuency of local reactions, by age, in vaccinated and control groups

Age group (years)	Vaccinees		Controls	
	No.	Per cent	No.	Per cent
1–5	21	8.8	12	5.4
6–11	110	20.4	29	5.9
12–19	49	29.3	15	9.0
20–39	133	32.1	31	7.0
40 and over	65	25.3	17	7.1
All ages	378	23.4	104	6.7

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1

REACTIONS TO MONOVALENT ASIAN INFLUENZA VACCINE, 1957

PERCENT OF 1617 INFLUENZA VACCINEES AND 1563 PLACEBO CONTROLS HAVING VARIOUS CONSTITUTIONAL MANIFESTATIONS ACCORDING TO TIME OF ONSET

	Clinical Manifestations	Onset Following Inoculation			
		< 24 hours		24 – 72 hours	
		Vaccine	Placebo	Vaccine	Placebo
Fever	≥ 100°F. (Oral)	3.8	0.8	0.8	0.6
	99–<100°	1.5	0.4	0.2	0.3
	"Feverish"	1.4	0.1	0.2	0.2
	Total	6.7	1.3	1.2	1.1
	Headache	4.8	1.6	0.4	0.9
	Malaise	4.1	1.1	0.7	1.5
	Nausea	2.2	0.6	0.4	0.4
	Chills	1.4	0.6	0.1	0.1
	Muscle aches	0.9	0.3	0.4	0.2
	Vomiting	0.7	0.0	0.2	0.0
	"Stomach ache"	1.0	0.6	0.4	0.6
	Coryza	0.9	1.0	0.7	1.0
	Sore throat	0.9	0.6	0.2	0.6
	Dizziness	0.8	0.7	0.2	0.1
	Fatigue	0.6	0.3	0.2	0.1

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time so we are safe.

I am just taking the chair temporarily while Dr. Kendrick gives her paper.

Now we're starting into a different phase of our subject on clinical responses. We talked about the reactions of the children, and now we'll talk about their antibody responses.

Dr. Kendrick certainly needs no introduction. We would call her the "Queen of Pertussis." (Laughter)

Dr. Kendrick.

DR. KENDRICK: Well, the subject of circulating antibodies is a pretty old one, and you're not going to hear anything new. We're going to be just stirring up some old problems instead of giving any answers.

The answer with respect to one infective agent, host antibody system, does not necessarily apply to another.

If we were talking about response in diphtheria, where we have a strict toxin/antitoxin response, in which the disease is caused by this exotoxin, and the measurement of the antibody unquestionably has something to do with protection, that's perhaps quite a different thing from talking about an antibacterial immunity in a disease such as pertussis where the pathogenesis is not so clear. The basis for the disease is a little indefinite at times.

We are not going to take time for an exhaustive

review of the literature on this subject. I think that you're all familiar with a good bit of the literature on the possible relationship of circulating antibody and protection.

Just to refresh our memories, I recall that in the '30's Lucy Mishulow in New York compared some tests with complement fixation, agglutination, and the mouse protection test, and in this test she was using intra-abdominal injections with a mucin-treated culture, and in 38 cases of whooping cough she had 30 that gave a positive protection, five doubtful, and three negative.

And among the 30 that gave the positive protection she had 18 that gave a positive agglutination.

She came to the conclusion that the protection test was the more significant one.

Later, Sauer found what he considered a relationship between complement fixation and protection in a group of 89 infants at or below three months of age; 27 per cent were positive in complement fixation compared with 70 per cent in an older age group.

And he found in those two groups a difference in attacks of whooping cough following definite exposures. He gets seven times as many attacks in the group of children with the low complement fixation.

Miller a little later, working with different

circulating antibody tests, studied agglutination. He studied opsonic test, also complement fixation. He did periodic agglutination tests during a five-year period in 554 children, and during the period of observation there were 74 definite exposures indoors, and there were ten cases of whooping cough.

The pre-exposure titers of these ten varied from zero to 160, and among the 69 who escaped the last agglutination prior to exposure varied up to 2560, and 46 had titers of 320 or more.

These observations suggested to the authors that, "Whereas immunity may exist in the absence of demonstrable agglutinins, susceptibility does not occur in the presence of agglutinins in high titer."

And about this time Saco was doing work, and he came to use the term, as I recall, "protective titer." He considered that if there were a titer of 320 that there was protection.

Of course, when you come to talk about titers you have to stop to talk about technique and all the rest, which we can't do.

Winter studied the development of antibacterial, antihemagglutinin, and mouse protective antibody in whooping cough in convalescent children, which gives us some notion of what happens in the disease itself.

She used the intranasal test, and she came to the conclusion that the protective test was the one that had some relation to immunity, and that the others were not necessarily related.

Now, several authors have reported on other methods. The use of opsonins has been reported by several authors.

We did some work with that in our laboratories in Grand Rapids, and we might just stop for a slide. (Slide 1)

How am I doing?

DR. PITTMAN: Fine. Fine. Take your time.

DR. KENDRICK: This just gives an idea of the response both in disease and following vaccine. Here are eight tests. These are the same eight children that have been followed over a period of several years.

This is a group, not the same children, but these are cases of whooping cough. And this shows the rise of the opsonic titers, rapid rise, and then a little falling off, not much falling off, you see, in six months.

This high point is at two months, and then falling off at one year, but a level-off so that we still have these measurable titers after the three years.

In the next slide/(Slide 2) these are figures from our study on the booster response, so that we have here the test before and the test after.

I'm not going into the details of the timing, but those tests before the booster varied from a few months up to five years following the completion of the primary series of injections.

And if we just briefly look, we can see that, as far as the tests before and after, the ones before fall in this low range, and these are index figures to represent the strength of the reaction, 77 per cent in the low before and only 20 after, because they have moved over into the area of higher reaction.

So that these are the stronger reactions, from moderate to strong.

Eighty per cent of them were in that range after the immunization, the booster injection. And that booster injection, by the way, was half as much as was being used in the routine procedures.

This was an experimental thing, and we thought that a smaller dose was possible.

Now, undoubtedly, the agglutination test has been used more than any other. It's relatively simple, far less time consuming and expensive than some of the others.

However, we mustn't be misled by the apparent simplicity. For example, as more information has been gained about the culture B. pertussis we know now that due consideration must be given to the selection of the

test antigen.

B. pertussis is not the ~~serologicai~~ homogeneous group serologically that we used to think.

So that if we choose a culture for our test antibody which does not have coverage for the cultures that we're interested in at the time, then we're bound to get some false negatives and be misled.

And also it's an interesting thing that sometimes a culture which stimulates agglutinins very well is not itself a very good antigen for detecting agglutinins.

Our friend 18323 stimulates agglutinins beautifully, but it's not as good an antigen for testing for agglutinins as certain other cultures like 10536 or some others.

The basic concern, of course, is: What is the relation between agglutination and protection?

I would like to show a few slides just quickly to illustrate how we tried to approach this some time ago.(Slide 3)

What we thought would be interesting would be to test the blood of mice that had survived infection, those that did not succumb, and see what their titers were, what their agglutination titers were.

Here we have a whole cell antigen, reference 10, which was a reference antigen to Grand Rapids. This is from a challenge experiment in Grand Rapids with Dr.

Eldering. The whole cell antigen. And this is cell-free (indicating slide). Pillemer type cell-free antigen.

And S/T, survivors over total, you can see here. And these survivors were bled, and the agglutinin titer-- We wanted to have a group of six to bleed in six cases, but you see you can't do that if they don't survive.

Anyway, we did get our tests. And we have a negative, a negative, 1 to 32, negative, negative, 1 to 64. That is, this Pillemer antigen did produce agglutinins in the heavier dose.

And here all the way through. And we have done many experiments. The .06 dose, .06 billion, the first small dose, did not elicit agglutinins. The three-tenths, the mid-dose, did a few in some experiments. In this one, none. And the 1.5 will produce the agglutinins in a large percentage.

Next, please. (Slide 4)

Here we have a summary of 21 antigens. And the total number of mice involved. Survivors. And s/T. And here is the agglutination test in the survivors.

Now, positive 1 to 4 to 1 to 128, just to abbreviate this. There were no positives among these mice with the small dose. Thirty per cent here and 58 per cent here.

But it might be well to point out that these 56

mice and these 126 were just as much alive as the 211 where they had the positive agglutination.

Next, please. (Slide 5)

Here we have a similar type of experiment except that we are varying the interval from vaccine to challenge or bleeding.

Now, we have here a group of mice that were not challenged to bleed, in order to make an estimate of what the agglutination titer was at the time these mice were challenged. And we have here all were negative as far as the agglutination was concerned in this experiment at the time of challenge.

But we have here 70 per cent surviving, 64, and 16, depending on the intervals.

All right. Next one. (Slide 6)

This is an attempt to carry this over a longer period of time from five-tenths of a day, 1, 2-1/2, to 34. We thought perhaps we would demonstrate David Evans' early inhibition here but we didn't seem to.

These are the agglutination titers of the unchallenged mice, the controlled, unchallenged.

And here after the 34 days you notice we have the higher titer, 512, and the titer starting at six days as 1 to 4, a very small titer.

But notice we start having survivors, a few at one

one day, and then at two and a half days, three, four. Seventy-five per cent of them survived after four.

This was with a larger dose, you see, two billion dose. And 94 per cent after four days, a hundred, a hundred. So that there again we can see that we have protection before we have measurable agglutinin. That's all.

Now, we had a little different approach in the next experiment which we will show in just a moment.

Time?

DR. PITTMAN: You just go ahead.

DR. KENDRICK: No. Is somebody helping me keep time?

Just a few minutes.

(Slide 7)

Then in the next one we attempted to get the same agglutinin level by two different means. In a group of mice we got to a certain level with active immunization, injection of antigen. In the other group we attempted to get the same level of agglutinin as we did with the active, only by the injection of hyperimmune mouse serum, and then thought it would be interesting to compare the results with that.

These are the vaccine-injected. This is the antiserum. And we got titers as you can see, pre-agglutinin titers.

These are the controls for bleeding so they were not challenged.

128 pre. 512 here.

And the vaccinated mice were injected with normal serum to control that factor.

Now, the titers at these varying times you can see. And they were not too far apart. If anything, they were a little higher with the passive.

And then these are the survivors over totals, which is 91 per cent survivors here against 59 per cent.

That was supposed to be significant to the 5 per cent level.

It looked as if perhaps there was some other factor in the active immunized group besides the antibody measurable agglutinin.

All right. We can have the lights, please.

In these different agglutination tests it would seem that we have demonstrated protection certainly in the absence of measurable agglutinin. And we would deduce from this for one thing that if it were possible to separate the protective antigen from the agglutinogen completely that the agglutination test would be pretty useless.

We have tried to see whether we'd get any different kind of a measurement by using the FA antibody

technique, but I don't think we will have time to go into that.

I'd just like to say we are working on it. We have no indication that we are measuring anything too different than we are with agglutinins, although in some of the experimentalwork it is suggested that perhaps it does measure something different.

But it is something to continue working on. There are many problems involved in it. And we're getting too many positive FA's in children before vaccination. But we're coming to the conclusion that there are two factors involved in these tests, one the specific factor related to the pertussis/antipertussis component and another factor that may perhaps be related to the globulin/anti-globulin.

We think it's very possible that it will be necessary to accept this fact and start measuring the FA in the children at a higher level than we start measuring in the agglutinin.

I don't think we'll have time for those slides.

The first impression is that the FA is a little more sensitive, and it probably is, but we must pursue this problem of specificity further before we can know just what the place of the FA is.

Now, in summing up, experience of many workers

supports the value of the serologic tests for circulating antibodies as an indicator of response to an injected pertussis vaccine.

While agglutinin per se is not the protective antibody, it is elicited in general in parallel, and we need to remember that the experience we have had to date is mostly with the whole cell culture, and current research is directed toward separation of the essential antigen from the agglutinogen.

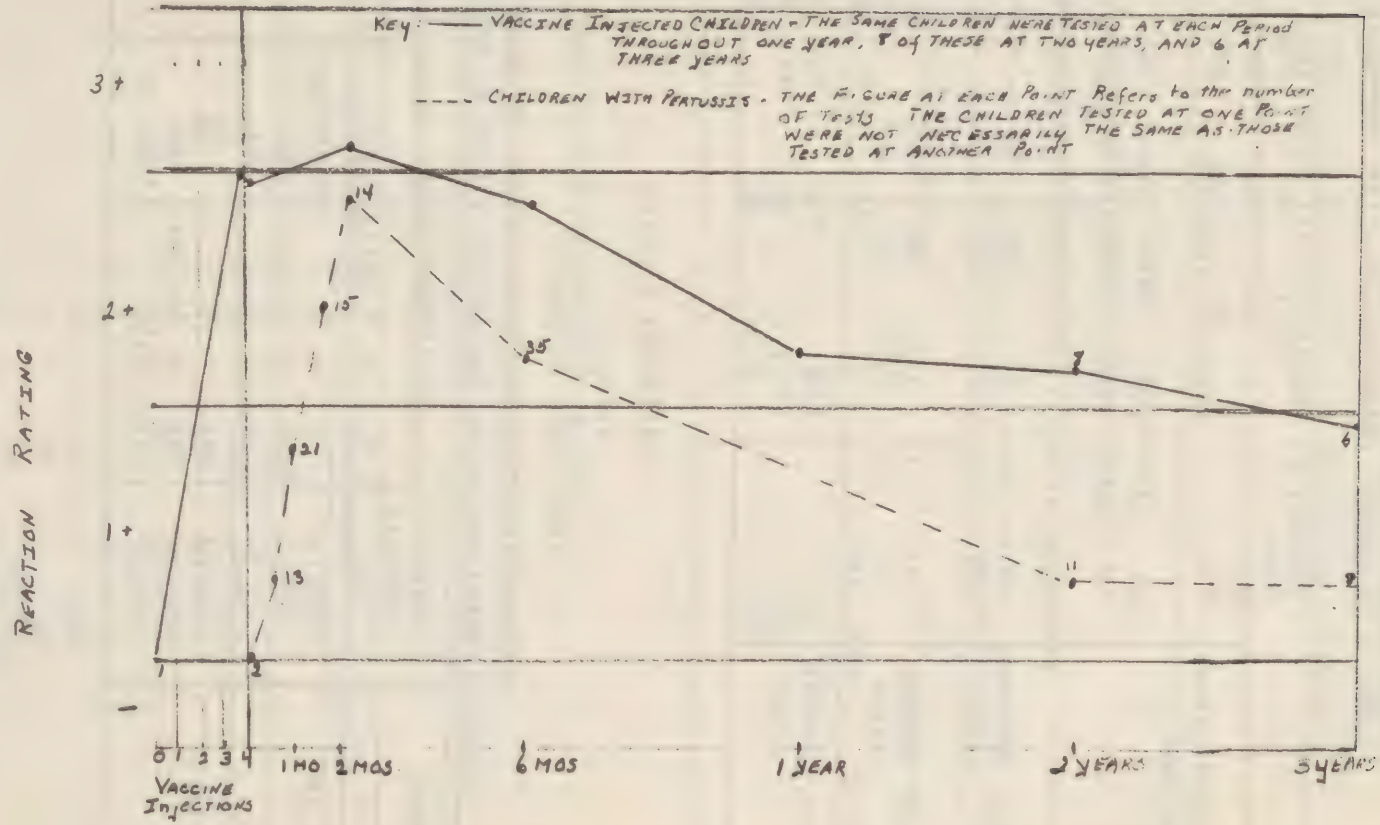
Now, if this is accomplished, we must reevaluate all of these antibody tests in terms of the newer antigen. If agglutinogen-free essential antigen becomes available, will it stimulate development of complement fixing antibody? Will this antibody act as an opsonin? Will it coat bacteria and be subject to stating by FA techniques? Will the antibody be present in sufficient concentration in infants that have had vaccine in order to express itself as a precipitate by diffusion?

And it would seem that as a reference against which to judge all these things certainly the essential thing we need is a protection test well standardized, one that can be repeated by different workers, and to act as the reference.

Maybe we'll hear about all these answers as the meeting progresses.

CHART 4 (Slide 1)

THE OPSONIC REACTION IN THE VACCINE-INJECTED CHILD COMPARED WITH THE CHILD WITH PERTUSSIS



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(Slide 2)

Table 3

OPSONO-CYTOPHAGIC REACTIONS IN CHILDREN BEFORE AND AFTER A "BOOSTER" INJECTION OF PLAIN PERTUSSIS VACCINE:

Summary of results at all intervals

Before and after booster dose		Range of reaction							Totals
		26-50	51-100	101-150	151-200	201-250	251-300	300& over	
Number	Before	5	50	50	39	31	12	0	187
	After	0	1	12	26	74	73	1	187
Per Cent	Before	3%	27%	27%	20%	17%	6%	0%	100%
	After	0	.5	6	13	40	40	.5	100
	Before			77%				23%	100%
	After			20				80	100

(Slide 3)

Slide 1

(Exp. 481, in part)

PERTUSSIS VACCINE PROTECTION TESTS IN MICE:
Survival Rates and Serum Agglutinin Titers in Survivors

Antigen	Graded dose in bil.	Challenged Mice				
		T	S	ED/50	S/T	Agg. titers S
Ref. 10	0.06	14	2		14%	-
	0.3	14	5	0.37	36	-
	1.5	14	13		93	1:32
Cell-free P Sd	0.06	14	1		7%	-
	0.3	14	7	0.30	50	-
	1.5	14	13		93	1:64

Slide 2 (Slide 4)

SUMMARY OF PROTECTION TESTS WITH 21 PERTUSSIS ANTIGENS: Survival Rates and Serum Agglutinin Titers

Graded doses of antigen (Bil.)	Challenged mice			Agglutination test: Survivors Positive 1:4-1:128
	T	S	S/T	
0.06	289	56	19%	none
0.3	203	126	43	30%
1.5	284	211	74	58%

Slide 4 (Slide 5)

PERTUSSIS VACCINE PROTECTION TEST IN MICE

Survival Rates and Agglutinin Titers in Survivors

Interval: Vaccine to challenge or bleeding	Vaccinated mice - One injection of 1.5 bil.					
	Challenged				Not Challenged	
	T	S	S/T	Agg. S	Chal. Date	End of Exp.
15 days	23	16	70%	1:16	(20)-	(19) 1:16
10 days	25	16	64	1:16	(18)-	
5 days	19	3	16	1:16	(17)-	

Slide 3 (Slide 6)

PERTUSSIS VACCINE PROTECTION TEST IN MICE:

Survival Rates and Agglutinin Titers in Survivors

Interval: Vaccine to challenge or bleeding	Vaccinated mice - One injection of 1.5 bil.			
	Not challenged: Agg. titers on chal.-date	Challenged		
		T	S/T	Agg. S
34 days	1:512	10	90%	1:512
18	32	15	100	512
10	8	14	100	256
6	4	16	94	256
4	-	16	75	8
3	-	16	75	128
2.5	-	16	44	32
1.0	-	15	13	No test
0.5	-	16	0	No test

Slide 5 (Slide 7)

PERTUSSIS VACCINE PROTECTION TEST IN MICE: Survival Rates in Relation to Agglutinin Titers Achieved by Injections of Vaccine and Specific Antiserum, Respectively.

Groups of Mice Treatment		Controls for bleeding: Agglutinin titers Days after challenge - date:				Challenged Mice		
		Pre	4	8	12	T	S	S/2
VACCINE (2 inj.)	Chal.		64	64	128	22	20	91%
	Not chal.	128	64	128	64			
ANTISERUM (3 inj.)	Chal.		256	64	128	22	13	59%
	Not chal.	512	256	128	64			

Note: Normal rabbit serum was given to the vaccine-injected mice: in same amounts and at same time as antiserum.

(Applause.)

We're certainly glad to have with us Dr. Cohen, and we are going to have the pleasure of hearing him now. You will see from your program that he will speak on establishment of and need for a freeze-dried hyperimmune antipertussis serum as reference for the passive intracerebral mouse protection test.

DR. H. H. COHEN: In the past few years the use of hyperimmune human gammaglobulin for specific prophylactic and therapeutic purposes has aroused general interest and gained wide applications. Moreover, it may yield important clues to potential methods of active immunization.

In the case of poliomyelitis, for example, protection could be obtained by means of specific gamma-globulin. ~~This~~ There was at that time an indication that active immunization would finally prove to be successful.

The reverse is also true. If, as is the case in pertussis, the potency of a vaccine of known activity for man can be predicted in a laboratory test, vaccines of different potencies should stimulate different amounts of antibody in humans.

In pertussis the efficacy of human pertussis gammaglobulin is controversial. The high mortality in unimmunized children under six months of age makes

an objective evaluation of its prophylactic and therapeutic value highly desirable.

Some authors report favorable results in young infants prophylactically as well as therapeutically.

In a more recent controlled experiment involving 50 family contacts carried out by Morris and MacDonald, results were different. These authors gave 2-1/2 milliliters of two different preparations of hyperimmune antipertussis gammaglobulin, a placebo being given to the control group.

No evidence of any prophylactic or therapeutic effect was obtained. In no trial, however, was potency was assayed by measuring quantitatively the antibody content against the antigen which is generally held responsible for human immunization -- that is, the protective antigen assayed in the intracerebral mouse protection test.

In this paper data are given of the establishment of a dried reference rabbit serum in the passive mouse protection test, to which a unitage was assigned.

With the aid of this serum a number of human gammaglobulins of different origins could be gauged in the passive mouse protection test. Moreover, an impression could be gained about the amount of antibody appearing after vaccination or natural infection.

The first point is the establishment of a dried reference preparation and a comparative assay with the NIH reference in our Institute and in the National Institutes of Health here in Bethesda carried out by Dr. Pittman.

We hyperimmunized rabbits with a killed pertussis vaccine. After completion of the injection scheme, they were bled. The sera were pooled and lyophilized in one milliliter amounts. Arbitrarily a unitage of 60 per milliliter was assigned to this Dutch reference serum.

It was compared in the passive protection test with the provisional NIH reference serum in the National Institutes of Health in Bethesda and in the Rijks Instituut.

The NIH reference serum is also of rabbit origin but refined and concentrated before freeze drying.

Both laboratories injected three groups of mice, each group with a different serum dilution. Dr. Pittman gave 0.2 milliliter serum dilution subcutaneously four hours preceding the intracerebral challenge, while we injected the final dose in a 0.5 milliliter volume intraperitoneally 18 hours before challenge.

(Slide 1)

In Table 1 the results of both laboratories with the U.S. standards are given.

Here you see the Bethesda results in five experiments. The log ratio between potency. Here you see the Utrecht experiments. The standard deviations. We used in each experiment more mice than were used in Bethesda so our standard deviation is a little bit smaller.

There was no significant difference in the slope of the lines.

Here is the ratio with one very low one. Here is the ratio in Utrecht. But there is no significance in the 95 confidence limits between these results, so we calculated the overall results, which is the American preparation being 5.1 as potent as the Dutch preparation and having, on the basis of 60 units, 306 units with the 95 per cent confidence limits you will find here, 126 to 738.

Thank you very much.

From the results in this table it may be concluded that on the whole reasonable agreement existed between both laboratories, with the possible exception of this one experiment in Bethesda. This value, however, lies within the confidence limits and was included in the calculations.

The second investigation we carried out was a potency assay of some lots of human hyperimmune gamma-globulin in the passive mouse protection test.

Can I get the next slide, please? (Slide 2)

You can see three preparations, two Dutch ones, one American one, and here a normal gammaglobulin. Here is the number of units in a milliliter.

The tests were for the Dutch preparations repeated one time. Agglutinin titers are given.

Here you see the protein content.

It seems that the USA preparation is slightly better because the protein content, as you will see here, is lower than the protein content of the Dutch preparations.

Thank you.

It can be inferred that the introduction of a freeze-dried reference serum of rabbit origin will make it possible to gauge protective antibody content of hyperimmune human antipertussis gammaglobulin. In this way it may contribute ultimately in assessing the value of these preparations for treatment of humans.

We did some investigations about a protective antibody content in sera of children after vaccination with DPT vaccines with a different potency of the pertussis component.

We have carried out a number of antibody

determinations in the blood of children who had been immunized with three injections of either of two DPT vaccines. Vaccine A was one of a series of vaccines of inferior quality. The average value of 13 lots of this vaccine was 4.8 protective units per total immunizing dose. Whereas Vaccine B belonged to a series of five lots of good protective value with an average of 19.4 protective units per total immunizing dose.

Protective antibody was assessed in the passive mouse protection test, and agglutinins were measured in a standard agglutination test.

Determinations were carried out before vaccination and four weeks after the third injection. In a number of cases a serum sample became available four weeks after a booster injection which was given one year after the primary series of injections.

In the samples taken before vaccination, neither protective antibodies nor agglutinins were found. The greater part of the sera taken after primary vaccination had a low protective antibody content, less than ten units in a milliliter. This made an accurate evaluation of the test difficult for two reasons:

(a) The sera with the low protective antibody content seem to respond in the passive mouse protection test with a low slope of the log dose response lines,

a greater number of mice than expected dying in the lower dilutions.

(b) The larger amounts of sera needed in these cases were not always available. It was therefore decided to rank the results in three classes -- smaller than one unit in a milliliter, one to ten units in a milliliter, and larger than ten units in a milliliter.

In the following table the results are given.

(Slide 3)

This is three injections.

This is the primary vaccination. Preparation A and B. And you see that there is no difference between both groups in the protective antibody content units per milliliter. The figures are exactly the same.

With the agglutinin titer there is a difference. You see the preparation A, of lesser quality, children respond less here than in the group B which is the better antigen.

Here is the average geometric mean agglutinin titer in group A, and here in group B. There is a clear difference.

Thank you.

The booster injection given after one year does not seem to influence the titer very much. The results after primary vaccination and after the booster are given in the following table. (Slide 4)

You see the children belonging to group A or B. Protective antibodies after primary immunization and after booster. See, there is not much difference with possibly one exception.

And here is the agglutinin titer of both groups, group A and B.

On the whole the titers are the same with the exception of here and again here (indicating) which gives a fourfold raise in agglutinin titer.

This child is very interesting, D44, because it suffered a light attack of pertussis between the primary series and the booster injection. We didn't know that but we heard it later. It's not clear whether the booster acted as a booster on priming by the disease or the disease as a booster on primary vaccination.

Anyhow, this finding proves that babies are capable to form considerable amounts of protective antibody.

In this respect the findings given in the following table and the next figure are interesting.

May I have the following table? (Slide 5)

Here we see a child who actually had pertussis. It was injected after the disease, seven weeks, with three injections of DPT vaccine. This is after the disease. This is after one, two, three DPT vaccine.

Here are the results of the agglutinins and the passive mouse protection.

You see here only three of 15 surviving after the disease. So not much protective antibody formed. But immediately after one DPT injection we see that the number of protective antibodies is rising to about 16 per milliliter. The agglutinin titer is rising very much.

And you see after the second it is on the whole the same, and it doesn't change any more after the third injection, agglutinin titers remaining the same.

And I wouldn't say this is a significant difference.

(Slide 6)

In the next figure you will see the same situation. The black points or triangles are protective antibodies or agglutinins. After the disease. This is the time after the disease. And all these children got one booster injection of DPT. One injection.

And here you see that both of them that are open dots and open triangles, that especially protective antibodies but also agglutinins raised to a fairly high value after one injection of DPT which is given in children recovered from the disease.

After priming by the disease itself, the vaccine injection gave a considerable booster effect especially on the protective antibody content. This is consistent

with the fact that pertussis gives lifelong immunity in practically 100 per cent of the cases, while the protection rate by vaccination with vaccines of good quality is estimated to lie only between 85 and 95 per cent.

Our general conclusions are the following:

Establishing a freeze-dried antipertussis rabbit serum as a reference serum, to which a unitage was assigned, makes it possible to evaluate in the passive mouse protection test protective antibody content in human hyperimmune antipertussis gammaglobulin.

A comparative assay in the USA and in the Netherlands between the Dutch reference preparation and the NIH standard preparation gave comparable results.

It should be kept in mind, however, that results obtained in the mouse protection test show relatively wide variations. The results, semi-quantitatively as they are, may, however, contribute in assessing the prophylactic and therapeutic value of hyperimmune antipertussis human globulin in the field.

Moreover, if such an effect exists, the number of units may be determined which should be injected in various circumstances.

In our hands the test had one limitation. It was difficult to measure quantitatively the amounts of antibody lower than ten units in a milliliter in children's

sera, as especially lower dilutions did not give the expected protection.

In infants after vaccination, including the booster, and after recovery from the disease, only limited amounts of protective antibodies are formed.

Both factors may have contributed to the fact that no difference in protective antibody formation was found in two groups of children immunized with DPT vaccines of different qualities, after priming as well as after a booster injection.

Contrarily, a marked difference was found in both groups in agglutinin content.

The immunological system in infants is capable to form large amounts of protective antibody. This is shown by the fact that one injection of DPT vaccine after recovery from the disease has a considerable booster effect on protective antibody formation. This implies that the priming by vaccination in babies is far from optimal.

Development of a pure soluble protective antigen which can be used in concentrated form is therefore desirable not only to minimize harmful side effects but also to obtain an optimal immunization status in children.

Thank you.

(Slide 1)

Table 1

Logarithme values potency ratio USA reference serum. Dutch reference serum

	Exp.	Log.ratio	standarddeviation	Ratio	Number of units/ml in USA serum
Bethesda	1	0.957	0.66	9.1 x	546
	2	1.011	0.67	10.3 x	618
	3	0.460	0.71	2.9 x	174
	4	0.182	0.67	1.5 x	90
	5	0.368	0.67	2.3 x	138
Mean Bethesda (1-5)		0.596	0.30	3.9 x	234
Utrecht	6	0.757	0.40	5.7 x	342
	7	0.950	0.44	8.9 x	594
	8	0.760	0.42	5.8 x	348
Mean Utrecht (6-8)		0.822	0.24	6.6 x	396
Mean (1-8)		0.709		5.1 x	306 (95% confidence limits 126-738)

(Slide 2)

Table 2

POTENCY ASSAY IN PASSIVE MOUSE PROTECTION TEST AND AGGLUTININ CONTENT OF A NUMBER OF HYPERIMMUNE HUMAN ANTIPERTUSSIS GLOBULINS

Origin	Units/ml in passive protection test	Agglutinin titre	Protein content
Dutch I	87, 66	3200	8%
Dutch II	178, 151	6400	16%
U.S.A.	144	1600	9%
"Normal" gammaglobulin	< 5	not done	10%

(Slide 3)

Table 3

NUMBER OF SERA, SHOWING DIFFERENT AMOUNTS OF PERTUSSIS PROTECTIVE ANTIBODIES AND AGGLUTININS IN POSTIMMUNIZATION SERA IN TWO GROUPS OF CHILDREN, EACH GROUP BEING VACCINATED WITH THREE INJECTIONS DPT VACCINES OF DIFFERENT PERTUSSIS-POTENCY

Vaccine	Protective antibodies units/ml			Agglutinin titre			Geom.mean agglutinin titre
	< 1	1-10	> 10	< 160	160-1280	> 1280	
A	5	12	4	12	9	1	100
B	3	12	4	4	15	3	320

Table 4 (Slide 4)

INFLUENCE OF BOOSTER INJECTION ON TITRES OF PROTECTIVE ANTIBODY AND AGGLUTININS IN CHILDREN PRIMARILY VACCINATED WITH 3 DPT INJECTIONS

		Protective antibodies (units per ml)		Agglutinins	
Child no.	Vaccine	After primary immunization	After booster	After primary immunization	After booster
D 6	A	2	6	20	80
D 7	B	5	7	640	640
D 12	A	12	22	320	160
D 22	B	2	2	160	160
D 30	A	> 1 < 10	< 1	40	80
D 31	B	> 1 < 10	>1 < 10	160	320
D 33	A	< 1	< 1	80	< 20
D 37	B	30	19	640	640
D 44	A	> 1 < 10	51[x]	640	2560[x]
D 55	B	15	8	5120	10240

[x] Mild case of pertussis between primary immunization and booster

(Slide 5)

Table 5

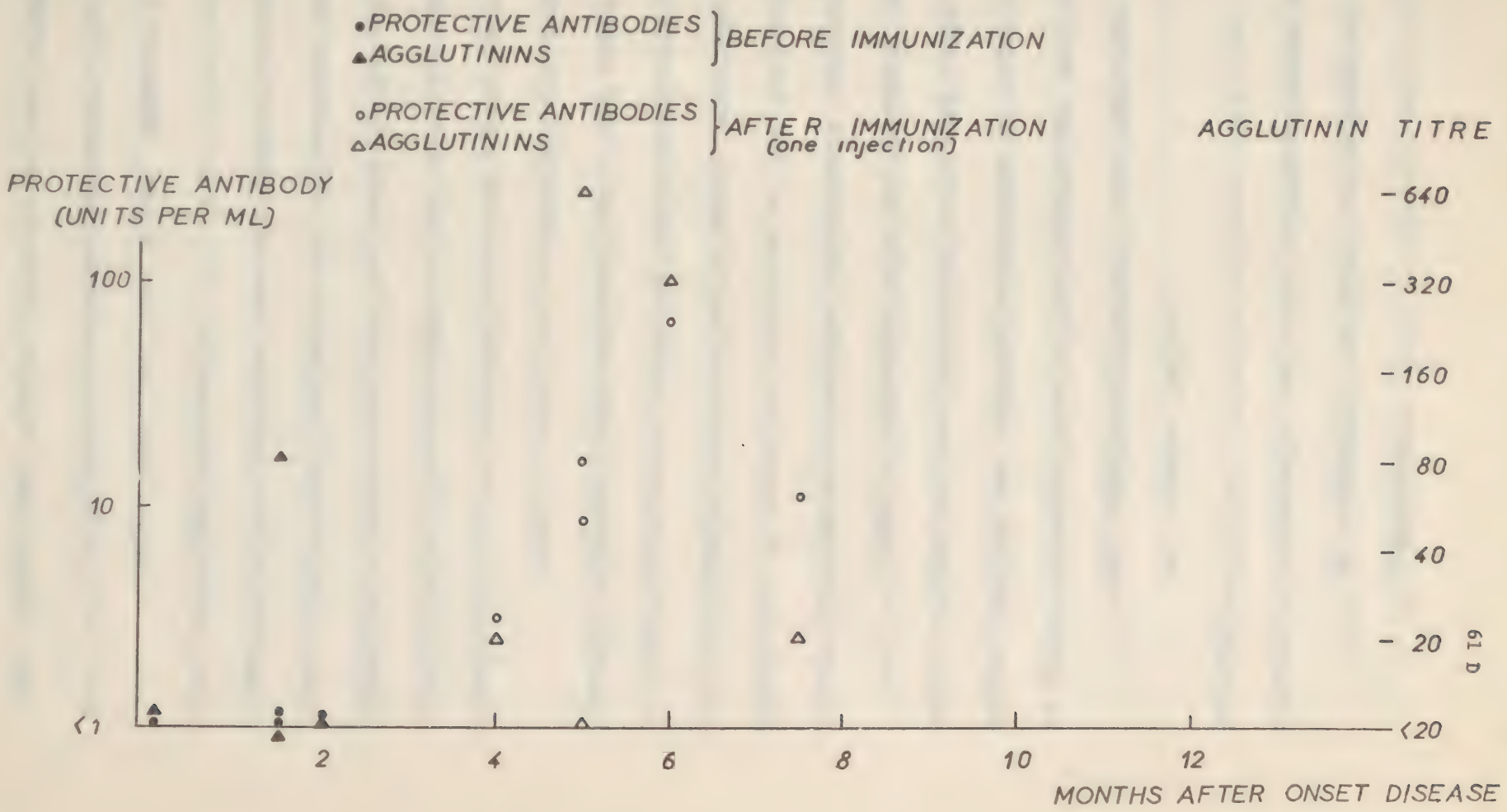
ASSAY IN THE MOUSE PROTECTION TEST OF 4 SERUM SAMPLES OF A CHILD 7 WEEKS AFTER THE DISEASE AND CONSECUTIVE DPT VACCINATION

Reference serum		Serum samples				
dose in ml	survival/total	dose in ml	survival/total			
			I 7 weeks after disease	II after 1 DPT dose	III after 2 DPT doses	IV after 3 DPT doses
0.05	14/20	0.125	3/15	12/20	17/20	13/20
0.01	8/20	0.025	3/20	6/20	12/20	9/20
0.002	2/20	0.005	1/20	1/19	4/20	3/20
ED 50	0.0203 ml		not calculated	0.0746	0.0190	0.0443
Units/ml	60		< 1	16	63	27
Agglutinin titre			80	640	640	640

(Slide 6)

FIGURE 1

INFLUENCE OF PERTUSSIS IMMUNIZATION ON TITRES OF PROTECTIVE ANTIBODIES AND AGGLUTININS IN UNVACCINATED CHILDREN WHO CONTRACTED THE DISEASE BEFORE THE AGE OF THREE MONTHS



(Applause)

DR. KENDRICK: We shall now proceed to the effects of Bordetella pertussis on tissue cells in culture.

Dr. Harriet Felton.

DR. HARRIET M. FELTON: Some years ago I happened to be at the right time in the right place with the right people and had the exciting experience of learning to watch antigen/antibody reactions in living cells.

I was in Galveston with Dr. Pomerat. He very kindly allowed me to use the magnificent equipment in his laboratory to study the reactions of the Bordetella pertussis and to see what could happen with the introduction of hyperimmune serum, after we had observed the cell reaction following the introduction of the whole organism.

Today I would like to show you the typical reactions that we found in the cells and to describe to you briefly the evolution of the machinery and the equipment that we used over the years and briefly to show you the final equipment that we used not with pertussis but as we advanced into this type of investigation with the staphylococcus.

May I have the first slide, please.

We started these experiments with the use of fetal human brain tissue. This is a fixed preparation

from a roller tube explant of fetal cerebellum. It shows the final effect of a 48-hour culture of a third-generation Kendrick strain.

The little granules represent the pertussis organisms.

Since this is a fixed preparation, we see the final effect of the conglomeration of the organisms as they enter the cell and come up against the nucleus.

May I have the next slide?

This is another fixed preparation that we just happened to catch showing the organisms coming in in a vacuole.

Of course, these are all fixed slides so that we just have one moment in time.

These cells are the supporting tissue of the brain, probably astrocytes.

May I have the next slide?

This is a photomicrograph of another cerebellum explant. We introduced the culture of pertussis, allowed it to be in contact with the cells for 48 hours, and then removed the suspension and introduced serum from babies, hyperimmune serum from babies, who had been routinely immunized.

This preparation shows the cells after they have had pertussis organisms in contact with them for 48

hours (indicating right side). And then serum from babies who had been routinely immunized. Serum had titer from 1 to 2560. This cell looks pretty much like it did before it had anything in.

On this side (indicating left side) we have a sister preparation which had the pertussis organisms introduced for 48 hours and then serum from individuals who had never had pertussis as far as we knew who showed no circulating antibodies and who had negative skin tests to the agglutinogen material we used to skin test with.

On this side (indicating left side) you see that the cell has pretty well disintegrated. The nuclei are distorted, and there is a vacuolization and general destruction of the cell.

Next slide.

This is another similar preparation, again on this side (indicating left side) showing the effect of the serum of the immunized babies, and in this side (indicating right side) the cells after they have been treated with serum that as far as we knew contained no antibody of any sort.

Next slide, please.

In the early work with this material and using these techniques we used explants of human fetal material as it became available to us. We had many things to do

lots of times when we didn't have this material available so we proceeded to try other kinds of tissue.

We found newborn kitten brain had very similar cell structures and reacted in very similar ways to the human material.

For several years we worked with the kitten brain using whole organisms, using killed cultures, and from our kind friends at Sharp & Dohme we began then to use the different antigenic components that were being separated out there.

We found slight variations on this general theme of cytopathology when we used the sonic extract of Bordetella pertussis in very low dosages. We could produce the damage practically like the one that you saw with the cell that had only normal sera.

We had an intermediate fraction which again in titrated doses would give us the same cytopathologic picture.

And, finally, we used agglutinogen as it was prepared free of toxin and found that we could run it through just as we could run through our nutrient fluid. It didn't bother the cells in any way at all.

We were very interested in this type of an experimental setting because you can hook all of these cell preparations up to microscopes with a camera hoisted on

top, and in this way we can have a continual permanent record of our experiment.

We found that in the process of carrying through our experiments we were able to have very precise points in time in which we could show predictable and very accurately reproducible cell damage.

As we went along we did this work with several antigens. Most of the time we used Dr. Kendrick's 18328 strain. We had some fun trying to figure out the dosage of organisms for a roller tube with several little tiny explants of brain tissue, but with some consultation we finally arrived at a dose that did give us very reproducible results.

We tried several types of serum again with known titer always checked by several laboratories, and with no exceptions we were able to reverse this cytopathologic picture in all of our preparations.

About the time that we were very interested in the work with the fractions, the staphylococcus came along, and we were sidetracked into working with this organism that was causing us a great deal more trouble than the pertussis was causing at that time.

And finally we were able to set up a system as an experimental tool using two microscopes that had a sort of binocular arrangement in the middle to put the

frames of the picture from each microscope on the same slide, and I'll just show you how we did that. I'll show you the result. And then at the end of the session, if anyone wants to see a picture of this machinery, I'll have it in the back of the room.

We feel that with this present instrumentation that there is a very precise experimental tool that can be used to study now cell strains, individual cells. With the advent of the Colter counter and very carefully regulated light sources, it is possible that there may be a better way to study the effects of these antigens and the antibodies either working synonymously with them or coming in after.

May I have the last slide? Just imagine this slide standing up. Here we have a single film frame. We have on one side the experimental material and on the other side the control material. In this way we can study a parallel experiment all the way through. It can be annotated for time and for the experimental items that are being used.

This does give you a permanent continuous record of changes that are happening in a situation that can be controlled to any extent that is within the realm of the investigator's ingenuity.

(Applause)

DR. KENDRICK: This is an area in which there has been very little work reported, the bacteria and cell culture work. Perhaps there will be more now.

The next paper is experience with a pertussis human serum neutralization test using intracerebrally injected mice. Mr. Paul Ensminger and Dr. C. G. Culbertson.

MR. PAUL ENSMINGER: Dr. Kendrick, Ladies and Gentlemen: The passive intracerebral mouse test for Bacillus pertussis neutralizing antibody in serum has been used in our laboratory for ten years. Experimentation with this test was at first started in response to the expressed preference of several investigators for such a test over agglutination and other methods.

Dr. Kendrick has already mentioned some of the references.

But, briefly, North, Mishulow and McGovern all found evidence indicating that agglutination, complement fixation, and opsonin titration tests probably did not truly measure the most important specific resistance factors in immune animals. These antibodies are not present in patients convalescing from whooping cough, as Mishulow found out.

In 1958 Standfast reported that the most important antibody responsible for protecting children appeared to be the antibody protecting mice against challenge by the

intracerebral route. Later investigations, notably by Preston and Te Punga, and by the British Medical Research Council, did not support these claims.

Preston and Te Punga found that agglutination could be correlated with immunogenicity of pertussis vaccines if prior adsorption of the serum with autoclaved and steamed cells was done.

In addition, the large amount of data collected by the British Medical Research Council indicated a correlation between agglutination, the mouse protection test, and the results of field trials.

Nevertheless, the neutralization of intracerebrally injected B. pertussis has served as an additional indication of immunity produced by injection of various vaccine preparations.

First slide.

We perform our passive intracerebral mouse protection test a little differently than I think has been mentioned here. This is the method that we use to perform this test.

We heat-inactivate serum and make suitable dilutions of the challenge culture, add two volumes of the undiluted heated serum to one volume of the challenge culture, mix, incubate 30 minutes at 4 degrees C., inject the mixture intracranially in the mice, and observe

for 14 days.

High serum titers with every species of immune animal sera tried were obtained using this serum assay method.

Next slide, please. (Slide 2)

This will show some of the typical results that are possible with this test using serum other than human. You notice we used five different animal species here.

We also used a whole cell and cell extract.

These LD_{50} values are number of virulent B. pertussis cells recorded. Over here, fold difference between pre- and post-immune titers.

This was 106 times difference between pre- and post-, and so on down the line here. Some of these are not too great here, and a few of these we just gave one injection of the antigen to so we didn't expect too high a rise.

Many of these sera were hyperimmune, accounting for the large increase in titer at post-immune level in most cases.

Some of the examples of the titers that can be expected when children's serum is used are shown in the next slide. (Slide 3)

This again is recorded as survivors over total number of mice injected at various levels of virulent B. pertussis and the pre- and post-immune -- LD_{50} and the fold

difference between pre- and post-immune LD_{50}'s.

And as can be seen, there are substantial differences between pre- and post-immune in most cases.

As mentioned previously, this test has been used in our laboratory mainly to test human serum, and altogether over 600 pair of children's serum samples have been tested by this assay method, with 73 per cent of the samples showing an increased post-vaccination titer.

Moreover, 45 per cent of these post-immune serum samples increased in titer by a factor of three or greater over the pre-immune titer.

The next largest amount of serum tested in our laboratory by this method has been with monkeys, and we tested over 140 serum samples from monkeys, and all but one post-immune sample increased in titer by a factor greater than three over the pre-immune titer.

The most bothersome problem connected with the test is the fact that the pre-immune or normal serum antibody titers are generally higher than the LD_{50}'s for normal mice injected intracerebrally with virulent B. pertussis with diluent instead of serum.

In the case of the children's serum samples tested, maternal antibody probably is the cause for the pre-immune neutralizing titers being higher than the control mouse LD_{50}'s.

In the case of the animal sera, the normal heat-stable components in serum evidently exert a suppressive effect upon the virulent B. pertussis cells.

The elevated normal serum titers in rabbit serum are not due to bronchiseptica antibody, as live cells of both bronchiseptica and parapertussis were used to attempt to adsorb the factors out of normal animal and human sera causing the elevated pre-immune LD_{50} values, but no diminution of the titers was observed after such adsorption.

Adsorption of normal and immune serum was done with live B. pertussis cells in order to determine definitely we were dealing with B. pertussis antibody.

Next slide, please. (Slide 4)

The procedure briefly used for this adsorption is on this slide, where we wash live cells three times, and to the sediment from the third washing we add our serum, shake slowly for 16 hours, centrifuge, repeat steps 1 through 4 again, and finally filter through a Millipore filter.

The results of these adsorptions were quite satisfactory, as all of the antibody was removed from the serum.

Next slide, please. (Slide 5)

As you can see, these are serum numbers

from patients that received this. Survivors over total to again. Here we went from a titer of 470/less than 32, post-immune, 15,000, down to 210, and so on down as you will see.

The results in this test are the averages of two tests done on this type of technique.

This same technique also has successfully removed the antibody in normal and immune rabbit serum.

In order to demonstrate the reproducibility of this test, multiple tests were run on the same serum on the same day. Both rabbit and guinea pig antisera were tested, which will be shown on the next slide. (Slide 6)

It can be seen on here this is merely Test Nos. 1 through 5. The LD_{50}'s. It can be seen in the rabbit antisera the variation is slightly less than twofold and on the guinea pig is slightly greater than twofold.

Another test to determine the accuracy of the serum assay test was done on immune serum at twofold dilution increments starting with undiluted serum.

Next slide, please. (Slide 7)

This shows the results of going from undiluted down to 1 to 16 dilution of serum, and we get a graded response here. This was done on three separate tests with approximately ten mice per serum dilution per experiment.

Two in vitro tests, agglutination and

complement fixation, were compared with this passive intracerebral mouse test in order to determine if any correlation existed. Over 250 paired children's serum samples were compared with the agglutination test.

In general, the agglutination test is hypersensitive. When agglutination titers and mouse protection titers were both done on patients' sera, a larger percentage of sera consistently showed agglutination titers.

Next slide, please. (Slide 8)

This shows the comparison of the intracerebral test with the agglutination test. These are five different groups of sera that we got, and some are whole cell and some are extracted cells. And in every case except one there were a higher percentage of agglutination positives than there were intracerebral positives.

However, there does not seem to be complete correlation between agglutination and passive mouse protection test.

Next slide, please. (Slide 9)

This presents some of the typical data that we have gotten comparing agglutination, complement fixation, and mouse protection test, and this is the fold difference between pre- and post-immune titers. And as you can see, there does not seem to be any correlation at all.

Here, for example, is one of the highest CF tests we got and one of the lower ones on the mouse protection test.

And you see the same thing on the lower part. This is with monkey sera, by the way.

Possibly this type of data could be improved if we used adsorption with steamed cells and autoclaved cells as Preston and Te Punga have done.

In summary, the passive intracerebral mouse test for B. pertussis antibody, consisting of incubating live virulent B. pertussis cells with pertussis serum and injecting the mixture into mice after a suitable incubation period, has been described. The test has been used for rabbit, rat, mouse, guinea pig, monkey and human sera.

Over 600 paired children's serum samples and over 140 monkey serum samples have been used in this intracerebral test. The pertussis antibody was adsorbed out of the system by treating the serum with live pertussis cells and later removing the cells,

Reproducibility test results were presented.

There was no correlation with the agglutination test and the complement fixation test.

Thank you.

(Applause)

(Slide 1)

METHOD FOR PERFORMING THE
PASSIVE INTRACEREBRAL MOUSE TEST

1. Heat inactivate all serum at 56°C for 30 minutes.

2. Make suitable dilutions of the challenge culture in 1 percent Trypticase (Baltimore Biological Laboratories). For example: Make five-fold dilution increments starting with 100,000 B. pertussis cells contained in 0.01 ml.

3. Add two volumes of undiluted heated serum to one volume of the challenge culture dilutions and mix.

4. Incubate serum-challenge culture dilutions mixture at 4°C for 30 minutes.

5. Inject intracranially into mice, 0.03 ml/mouse.

6. Observe mice and record deaths for 14 days. Do not count any deaths occurring in the first 72 hours.

(Slide 2)

Table 1

Some Serum Protective Titers in Animal Sera as Tested by the Passive Intracerebral Mouse Test

Serum Used	Antigen Used to Prepare the Serum	Pre-Immune LD_{50}	Post-Immune LD_{50}	Fold Difference between Pre- and Post-Immune Titer
Rabbit	Whole cell	970	100,000	106
	Cell extract	1,040	>100,000	>96
Monkey	Whole cell	1,430	300,000	88
	Cell extract	1,105	>900,000	>810
Rat	Whole cell	215	3,430	16
	Cell Extract	380	1,640	4.3
Mice	Whole cell	402	2,825	7.0
Guinea pigs	Whole cell	<160	7,700	> 48
	Cell extract	<160	16,600	>104

(Slide 3)

Table 2

Some Serum Protective Titers in Human Sera as Tested by the Passive Intracerebral Mouse Test

Patient No.	Survivors/Total Mice Injected — No. of Virulent B. pertussis cells injected per mouse					LD_{50}	Fold Difference between Pre- and Post-Immune Titer
	20,000	4,000	800	160	32		
692 Pre	-	-	1/10	6/9	8/9	315	11
692 Post	3/10	4/10	7/10	9/9	-	3,480	
843 Pre	-	-	4/9	9/10	10/10	675	3.2
843 Post	1/8	2/9	7/9	8/10	-	2,140	
985 Pre	-	-	4/10	5/10	8/10	275	8.2
985 Post	1/10	4/10	7/10	7/10	-	2,240	
4191 Pre	-	1/10	4/10	7/9	-	635	31
4191 Post	6/10	8/10	10/10	10/10	-	20,000	
4272 Pre	-	2/10	2/10	10/10	-	635	6
4272 Post	3/9	4/10	9/10	9/10	-	3,760	
4305A Pre	-	1/10	1/10	6/9	-	405	16
4305A Post	1/10	6/10	9/10	10/10	-	6,500	
4129A Pre	-	1/10	4/10	8/10	-	640	5
4129A Post	2/10	3/10	9/10	9/10	-	3,100	
4163A Pre	-	2/10	5/10	8/10	-	800	21
4163A Post	5/10	9/10	9/10	9/9	-	17,100	

(Slide 4)

PROCEDURE FOR THE ADSORPTION OF SERUM WITH B. PERTUSSIS

1. Wash live B. pertussis cells three times.
2. To the cell sediment after the third washing add sufficient serum so that 3×10^{10} B. pertussis cells are present.
3. Shake the serum-cell mixture slowly at room temperature for 16 hours.
4. Centrifuge serum-cell mixture at 13,000 RPM for twenty minutes and discard sediment.
5. Repeat steps 1 through 4 one time.
6. Finally, filter the adsorbed serum through a Millipore GS filter (0.22 u pore size).

(Slide 5)

Table 3

Adsorption of Human Serum with Live B. pertussis Cells

Number of Challenge Cells/Mouse	Survivors/Total Number Challenged									
	K5-1 Serum	K5-1 Serum Adsorbed	K5-2 Serum	K5-2 Serum Adsorbed	K6-1 Serum	K6-1 Serum Adsorbed	K6-2 Serum	K6-2 Serum Adsorbed	K7-1 Serum	K7-1 Serum Adsor
20,000	-	-	10/20	-	-	-	5/19	-	-	-
4,000	1/20	-	16/20	-	14/20	-	10/18	-	10/20	-
800	7/20	0/20	17/20	4/20	16/20	0/20	13/18	0/10	17/20	1/20
160	12/20	1/20	9/10	12/20	20/20	0/20	-	0/20	19/20	4/20
32	-	2/20	-	14/20	-	0/20	-	1/20	-	6/20
LD_{50}	470	<32	15,000	210	>4,000	<32	5,700	<32	3,440	<32

Challenge Control LD_{50} - 158

(Slide 6)

Table 4

Variation in Serum Titers within One Test Using the Passive Intracerebral Mouse Protection Method

Sera Used	Test No.	LD_{50}
Rabbit	1	40,000
	2	63,300
	3	48,200
	4	50,000
	5	49,200
Guinea pig	1	2,600
	2	2,560
	3	5,500
	4	4,000
	5	2,100

LD50 Variations Using Different Dilutions of Pneumonia Antiserum (Murphy)

Number of Challenge Cells	Survivors/Total Number Challenged				
	Serum Undiluted	1:2 Serum	1:4 Serum	1:8 Serum	1:16 Serum
2,500,000	14/30	4/30	-	-	-
500,000	25/30	18/30	12/30	-	-
100,000	26/30	25/29	18/30	14/29	10/29
20,000	28/30	30/30	20/30	21/30	14/29
4,000	27/28	30/30	30/30	27/30	20/29
800	-	-	30/30	30/30	28/29
160		-		30/30	29/30
LD50	1,700	1,000,000	237,000	77,200	43,200

7

8

9

DR. KENDRICK: Thank you.

We are building up more and more material for our discussion.

The next paper, the epidemiology of whooping cough in a small outbreak in Kent County, Michigan, was to have been given by Dr. Harold Lambert. In his absence Dr. Eldering, who has been very close to this study, will give the paper.

DR. GRACE ELDERING: Dr. Kendrick, Dr. Pittman, Dr. Murray, Ladies and Gentlemen: Just being a substitute absolves me from certain responsibilities.

The final test of any specific immunization program is its effect in the community on the morbidity and mortality of the particular disease. It is, therefore, of special significance to observe the status of whooping cough in Kent County and Grand Rapids, Michigan, a community where field trials of pertussis vaccine were conducted over a period of years and where immunization has been maintained at a relatively high level for a long time.

In addition, there has been a continuous use of culture methods in the diagnosis of whooping cough for over 30 years.

By way of background, Kent County, including Grand Rapids, has a population of approximately 373,000, while the

city itself is somewhat under 200,000.

Some idea of the trend of whooping cough mortality in the nation, in Michigan and in Grand Rapids may be gained from the tabulations in the first two slides which were borrowed from Dr. Kendrick. (Slides 1,)

These are total deaths for five-year periods starting 1932 and ending with the period 1957 to 1961. And we won't go into these, but you can see that it has dropped from approximately 5,000 per year to somewhat under 200 per year in the nation.

In Michigan there has been a similar drop.

In Grand Rapids, Michigan our last death from whooping cough was recorded in 1946.

The next slide, please. (Slide 2)

This shows selected years starting with 1920. And these are rates per 100,000.

In the United States starting 1918 to 1920 the rate was 11.7 per 100,000, or a total of 18,988 deaths, dropping down to 1958 to 1960 where the death rate is less than two-tenths per cent, or a total of 575 deaths in the United States.

Now, in Michigan there has been a similar drop in death rates per 100,000 starting in 1920 with the death rate of 13.9 and dropping now to one death in 1960 which is not calculable.

(Slide 3)

In the next slide, also Dr. Kendrick's, we show figures for Grand Rapids, Michigan, moving averages, five-year averages, for various age groups, under one year, one to four, five to nine, starting with 1930, and unfortunately only going to 1951.

These are adjusted for populations, and the figures could not be completed because we didn't have population figures.

We will just emphasize here that while the one to four year -- these are morbidity rates, not mortality -- were way up in the early years -- the one to four was way above -- these three age groups have approached each other here (indicating 1951), and it would be very interesting to see what happens from there on.

Now, for the year 1960 in Grand Rapids we had 13 reported cases of whooping cough. In 1961 there were four. But in 1962 in the spring we noted that we were having an unusual number of requests for whooping cough diagnostic cultures in the laboratory in Grand Rapids. We had quite a number of positives, and most of these seemed to be of the high school age.

Now, we had noted an apparent shift to an older age group in the whooping cough cases in our laboratory, but we're not epidemiologists, and this was really an impression. So that when this little outbreak appeared to

be starting, it seemed important to have an epidemiologic investigation.

We were very happy when Dr. Harold Lambert from our department in Lansing came over and investigated this outbreak.

This report concerns his study, and it has been very much abbreviated.

The 89 study families, including 474 persons, were selected because B. pertussis was found on culture from some member of each family. That is, it started in the laboratory, you see.

It should be noted that the study group does not include all of the reported cases in the area.

Dr. Lambert himself interviewed all the families using a questionnaire to obtain information concerning family roster, birth dates, dates of immunization, kind of vaccine, where and by whom the vaccine was given, and so forth.

For those who had symptoms of disease or a positive culture, a detailed case history was obtained, and corroborative evidence was sought from the physician and from the public health clinics and the school records.

Nearly all the specimens for examination for pertussis were taken at our laboratory on referral of the patient by his physician. We collected data by means of

nasal swab of Bradford, and examination was by conventional culture methods and also by the fluorescent antibody staining applied either to the slide made directly from the smear or slides prepared from young cultures.

(Slide 4)

In the next slide/we have the total study group listed by age and number and per cent attack -- that is, under one year there were 21 in the study and 16 of these contracted whooping cough, 76 per cent. ~~xxxxxxx~~

Note that in the five to nine year age there were a total of 71 individuals, 29 of them contracting whooping cough, 41 per cent.

But here in the ten to 14 year age group, 82 of them, 52 contracted whooping cough, or 63 per cent.

Note here also that in the age group 20 or more, 186, most of them parents, 39 contracted the disease, or 21 per cent.

There is an accrued attack rate of 42 per cent.

Now, if we exclude 156 persons with a history of a previous attack and three infants who received hyper-immune serum, the adjusted attack rate was 62 per cent, for whatever that's worth.

(Slide 5)

The next slide/shows the 211 persons who by Dr. Lambert's definition were vaccinated. That means that they had received at least three doses of pertussis vaccine. And this also shows the number and per cent attack.

If we group the 99 persons under ten years, the attack rate is 32 per cent. And with the 112 persons ten years of age or over, the attack rate is 59 per cent, or pretty close to twice the attack rate for the lower age group.

A brief note about vaccine and schedules is of interest. Some of the older children had received single pertussis vaccine or combined pertussis vaccine and diphtheria toxoid, but nearly all the younger children had a triple product. Only eight individuals had received a quadruple antigen.

190 of 211 vaccinated children -- that means those who had received three or more doses --received the first three doses between three and nine months of age. That is, 90 per cent of those had their first three doses in that time.

Of 156 who got a fourth dose, or a booster, 115, or 74 per cent, had received that booster between the age of two and a half and five and a half years.

And of 46 who received a fifth dose, 32 of them, or 70 per cent, received it between the ages of seven and ten years.

Now, an interesting finding relates to the number of parents who had the disease. 28 of 180 parents had whooping cough during this time.

Six of 146 parents who gave a history of previous attack experienced a second attack. And in our experience, if you look for them, you'll find many parents who were having a second attack.

Two children were also having a second attack of whooping cough.

Now, unfortunately, the first attack was not confirmed by culture in any instance that we could find.

(Slide 6)

The next slide is really the gist of the whole thing. This lists the 211 persons who had whooping cough -- no, who had had vaccine, three or more doses of vaccine. According to the interval between the last injection of vaccine and this episode in Grand Rapids this year, and the number and per cent who were attacked.

Thatis, those within four years, who had had their last dose of vaccine within four years, included 85 persons, and of those 19 or 22 per cent had whooping cough last year.

Of 62 persons who had an interval of four to eight years between their last dose of vaccine and this exposure last year, 30, or 48 per cent, came down with the disease.

And of those between eight and 12 years interval between their last dose of vaccine -- there were 43 -- 29, or 67 per cent of them, got whooping cough.

Now, there were 21 people who had their last dose of vaccine 12 or more years before last year, and, of those, 20, or 95 per cent, almost all of them, got whooping cough.

So here we have very precise correlation between interval between last dose of vaccine and contraction of the disease.

Data omitted here because of lack of time include those relating to severity and to laboratory findings.

One point of interest is that nearly one-fourth of the laboratory-confirmed cases were not diagnosable clinically. That is, they had a cough for one week or less. Four of these were in the unvaccinated category, less than three doses. Some of them had had some vaccine. And 15 were in the vaccinated group. Two of the latter had no symptoms at all, which seems to fulfill the criteria for transient carriers.

In summary, then, data relating to 89 families in which whooping cough occurred in 1962 in Grand Rapids have been given. There was a definite correlation between the interval from the last dose of pertussis vaccine to exposure and contraction of the disease.

It is suggested that further studies of the interval factor be carried out in other geographic areas.

Thank you.

(Slide 1)

Whooping Cough Mortality: 1932-1961

5 Year Periods	Number of Deaths		
	U.S.A.	Mich.	G. Rapids
1932-1936	24,718	732	14
1937-1941	19,496	474	5
1942-1946	10,778	286	4
1947-1951	5,896	143	0
1952-1956	1,778	30	0
1957-1961	834	15	0

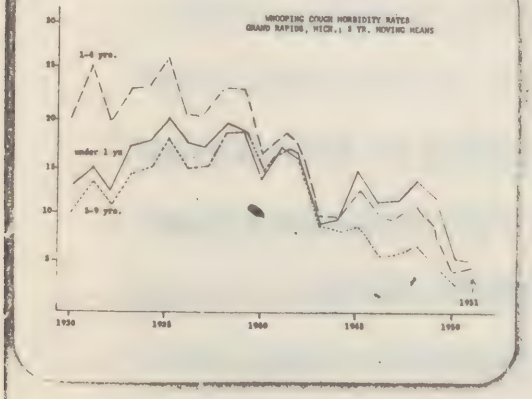
(Slide 2)

Whooping Cough: Michigan and U.S.A.
Selected Death Rates per 100 000

Michigan			U.S.A.	
Year	Number	Rate	Years	Rate
1920	511	13.9	1918 - 1920	11.7
1930	176	3.6	1928 - 1930	5.5
1940	59	1.1	1938 - 1940	2.7
1945	36	0.7	1943 - 1945	1.7
1950	28	0.4	1948 - 1950	0.7
1955	8	0.1	1953 - 1955	0.2
1960	1	0.0	1958 - 1960	<0.2

1. Number of deaths: 18 988 ② 575

(Slide 3)



(Slide 4)

Total study group by age and by number and percent attacked

Age Group	Number	Attacks	
		Number	Percent
< 1 yr.	21	16	76
1 - 4	56	29	52
5 - 9	71	29	41
10 - 14	82	52	63
15 - 19	58	32	53
20 or more	186	39	21
All ages	474	197	42

(Slide 5)

Vaccinated individuals by age group, and number and percent attacked

Age	Number	Attacks	
		Number	Percent
< 1	5	2	40
1 - 4	38	13	33
5 - 9	56	17	30
10 - 14	62	38	61
15 - 19	45	24	53
20 or more	5	4	80
All	211	98	46

(Slide 6)

Interval between last pertussis vaccine injection and exposure related to number and percent attacked

Interval in years	Number with vaccine history	Attacks	
		Number	Percent
0 - 3.9	85	19	22
4 - 7.9	62	30	48
8 - 11.9	43	29	67
12 or more	21	20	95
All intervals	211	98	46

(Applause)

DR. KENDRICK: Thank you.

We are now coming to the discussion part, and I wonder if we would just stand up and if the people who have taken part in the program would perhaps come up to this table and then as the people get up for discussion will you identify yourself, please, tell who you are, where from, and direct your question to the person from whom you would like a reply.

And will Dr. Brigham take the chair as leader of the discussion.

DR. GEORGE D. BRIGHAM: I'll just start off a little bit here by saying that as a manufacturer it's always a concern to us about reactions, and, of course, as a producer, the reactions always come to me. You know I am the last man you can come to. (Laughter) They pass the buck. And as all my competitors and friends know, that's what happens.

And in regard to this reaction, we have been very fortunate I guess. At least our records don't show too many as far as fatal reactions. I went back over the records using triple aluminum vaccine, and we have none reported there.

When we got to the triple aluminum phosphate we had one convulsion and one fatality.

And in the quadruple vaccine we now have one reported as far as the mental reaction occurring.

I think we all know that when you put out a new product you always get quite a few reactions, and then as the product has been used it drops off during the course of the years. Or if you changed the form of the product the same thing will happen we have noticed.

And so that rather than bore you with a lot of figures, I'd just like to mention that this does happen, we have found, at least in our manufacturing.

I was wondering in regard to this last paper here about vaccinating there -- this disease occurring in older people. I don't know how you would vaccinate these older people.

I might report that looking over our old records of reactions we had one that came in from a doctor who wrote in and said, "Boy, I got terrific reactions in three people."

And we wrote back and found out he had given them to a 17-year-old, 30, and 40-year-old persons.

So I don't know what would happen in this case here.

I think we have got to find a vaccine which is less reactive, because I don't think you can find one nowadays.

And as to the testing, I would prefer to let questions come from the floor here on these various tests, because I know very little about them.

Thank you.

DR. KENDRICK: Now, if you will identify yourself and direct your question to one of the persons concerned, we'd be very happy to have you fill this time as fruitfully as possible.

DR. F. T. PERKINS (Medical Research Council, London, England): We are getting down to this problem of reactions in immunized subjects because some pediatricians were saying that this was now causing more reactions than they had seen before.

In looking into this problem, however, it doesn't seem to be a problem that is coming up in the last two or three years. There was always a small background rate of reaction in these subjects following whooping cough immunization.

The pictures that are described by some clinicians are quite classical, and they divide into two groups, those who go into a complete coma and frequently in the physician's report you will have a sentence to the effect that, "I thought the child was going to die," and the other group are the ones where the children become almost hysterical and scream for a long period of time.

Now, this is occurring in a very small number of children. Nevertheless, the reactions, apart from local soreness at the injection site, those that are obviously going to have some brain involvement are divided into those two groups.

I would like to ask Dr. Christensen when he was describing in his survey where there were 21 cases, how many injections would have been given. Is it known how many injections would have been given over this period of time so that one could calculate an incidence of these reactions in your area of study?

DR. CHRISTENSEN: I know of no way you can arrive at that figure. I have tried and tried to decide whether it would be justifiable to put down known numbers of children born in this country during that length of time and put the number of reactions reported over it. But I don't think this would be fair either.

First of all, all the children aren't immunized.

Secondly, I'm sure their reactions-- Well, I'm not sure but it seems to me likely there are reactions occurring that aren't reported or that you won't pick up in a survey of this type.

After all, we talked to only 104 institutions. Most of the injections in this country I'm sure are given by private physicians. If a child had a reaction of this type

he wouldn't and in one of the so-called research institutions.

So I can't assign any figure for frequency or incidence.

DR. I. S. DANIELSON (Lederle Laboratories, Pearl River, New York): I was interested in Dr. Cohen's report where they used two different vaccines, one he admitted very poor, 4.8 units per total human immunizing dose, the other one up around 19.4. You didn't say anything about reactions there.

Was it noticeable between these two very far apart?

DR. COHEN: We didn't measure them at that time because we were primarily concerned about measuring antibodies. But as far as I am aware, we changed to a better vaccine let's say about six years ago, and this was our turning point, and the complaints -- but that is generally speaking -- of poor vaccines or better vaccines didn't make much difference.

And these are the cases, extremely rare cases, of encephalopathies which were reported. We had cases of encephalopathies before and we had them later, a small number of them, but we definitely had them.

DR. BRIGHAM: Dr. Barrett, have you seen any cases in Detroit on this older age group at all?

DR. BARRETT: No, but we have not encouraged immunization with pertussis-containing material beyond the age of six years.

As a department of health policy we suspend our preparations containing pertussis right at the age of six.

Our private physicians would tend to go almost completely with the State health department's recommendations, which are the same, and the Academy of Pediatrics.

I say that with tongue in cheek, because the one time that I chanced to come in contact with a physician that had started to use the quadruple vaccine, as they call it-- Now, this is an obstetrician, and he was doing this as a sort of a favor to a few of the families that he looked after. Normally he doesn't immunize. And the product was new at this particular time. This was about a year to 18 months ago.

And I asked him how he liked it, what he had to say about this. Any reactivity?

He said, "Oh, yes. I quit using it because of the enormous sore, swollen arms and the incapacitating effects" -- in these two or three out of the six children or so he had used it on.

I was curious about the age that he was immunizing, thinking that was it after first dose, second dose, third dose? It was only after the first. He started

that and quit.

And I was thinking about infants and pre-school immunization.

Then in further conversation he revealed he was giving it to children ten, 12, 18 years of age.

So I would like to have a pertussis antigen that could be used certainly up to the age of 15 and incorporate that along with our maintenance program against diphtheria, tetanus and polio.

DR. ELDERING: Madam Chairman, or Mr. Chairman, I should like to know the oldest age of person in whom an encephalopathy has been recorded.

When you talk about being afraid of reactions in the older age group, it isn't encephalopathies. Maybe they're just able to yell louder when they get older and complain worse. (Laughter)

DR. BRIGHAM: Could be.

DR. ELDERING: It isn't certainly encephalopathies you're afraid of.

DR. CHRISTENSEN: I don't know how frequently pertussis has been used in older age groups. If the incidence of these things is one in a million or one in a half a million, it might be that we wouldn't have picked them up.

DR. FELTON: Do you think I could talk about our

donors at the serum exchange?

DR. KENDRICK: Please do. That's a group with which we're much concerned.

DR. FELTON: For many years at the Children's Hospital at Philadelphia we have been hyperimmunizing donors, human donors, to prepare therapeutic pertussis serum. We used medical students, truck drivers, trolley car conductors. Most of these people have to use their arms in their work.

Over the years that I was there, I guess 14 or 15 years, we gave these boys a cc. of saline-suspended standard pertussis antigen once a month. Some of these men have been donors there for 20 years.

In the length of time that I was down there -- I don't know if they have had any trouble since -- we had no one with a severe systemic reaction, and very few with an arm that would be sore enough to keep them from doing their work.

Strangely enough, the men told us how to give this vaccine. When we had new doctors, we always had a little flurry of sore arms. But the men would say, "Doctor, give it to me back here" -- and they always pointed to a place about midway up the triceps back there where if you look in your anatomy book the nerves don't run. There is a sort of an empty space right back in here.

And if we gave the boys the shot where they said, there was just no difficulty, and these boys have been coming in for years and years and years.

And I think, beside the minor reactions, the thing that has fascinated us over the years is this crazy ceiling on the agglutinin titers. We have had one donor that reached a titer of 1 to 20,000, an occasional one that reaches a titer of one to 10,000, but the standard titer is some place around 1200 or 2560.

DR. HENRY B. DEVLIN (Parke, Davis & Company, Detroit, Michigan): You gave 1 ml. I wonder if you know about count or the type of product.

DR. FELTON: We used commercial material that was the standard saline suspension, and in the more recent years we have had to have it properly prepared. No aluminum. Just plain saline suspension. And it was the standard-- What did that used to run? I forget.

DR. BRIGHAM: Standard fluid?

DR. FELTON: Yes. 20 billion.

DR. ELDERING: We used ten.

DR. FELTON: I think we used 20 and probably came down to ten at the end.

DR. F. B. PECK, JR. (Eli Lilly and Company, Indianapolis, Indiana): We have a little information on some older children with the use of extracted antigen, not

with whole cell products.

In our clinical trial before this material was commercially available, there were a few children up to the age of about 12 years that received booster doses of this material. I would say there were probably on the order of 500 to 1,000 injections given by one investigator.

The incidence of his local or systemic reactions was approximately ten per cent.

Since that time he has given several hundred more injections to children anywhere from the age of 12 to 18 years with essentially the same reaction rate.

This is all we have clinical on right now.

DR. DEVLIN: It might be of interest to the group that we have been performing neutralization tests in mice too. We have a simplified test. It's just a one-level serum. In the 0.03 ml intracerebral dose we give 0.015 ml. of undiluted serum and 16,000 total count organisms. Then we do the control challenge, and, of course, we have no control serum except the pre-blood, and we do accumulated per cent deaths and then divide the control value by the serum value, and we have shown that we get factors greater than one would indicate there is a protective effect.

We find that these factors increase after the three doses, and also it's in the same direction as the agglutinin titer.

Of course, this was with whole cell vaccination.

We noted though there was a lot of monkey sera supposedly normal that would have a protective effect. And in very scanty work we could not adsorb this out with the challenge organism because your sera will go both ways. It will protect the mouse and it will also cause the challenge to be -- (inaudible).

DR. J. WHALEN (Pitman-Moore Company, Indianapolis, Indiana): I was wondering if you found any differences in strains in adsorbing these agglutinins, either your work or --

DR. DEVLIN: No, I'm sorry, we haven't. We have just used the one challenge strain.

MR. ENSMINGER: I haven't done any work on that at all.

DR. C. G. CULBERTSON (Eli Lilly and Company): Paul, if you don't use -- (inaudible).

MR. ENSMINGER: They, of course, have to be washed to get rid of any soluble antigen that might be in the solution.

DR. M. S. COOPER (Lederle Laboratories): In view of the interesting comments Dr. Cohen made, I'd like to ask him or Dr. Christensen or anybody who has an opinion on it as to whether there is a possibility that the encepha-lopathy after pertussis vaccination is not strictly

specific, specifically caused by a pertussis component, and whether there is a possibility that this hard-core, low-frequency reaction could have occurred due to the individuals themselves, whether traumatic experience of getting the injection or something similar to that.

DR. CHRISTENSEN: Whether the pertussis actually causes it or not and what the cause is, I think, are good points for discussion.

To me it doesn't seem like a sensitization phenomenon. Dr. Felton's experience with people who have had repeated injections over a long period of time would argue against the desensitization type of thing.

DR. FELTON: I have a lot more things. A lot of these donors went off to the war and came back, and the first place they came -- because they were low on money I guess -- was to the serum exchange. And in our-- I guess we were just too dumb to be afraid. We just started right up on their same vaccination schedule, and no problem.

DR. CHRISTENSEN: Whether there could be a toxic factor I don't know. We should be able to see dose-related phenomena in that situation. I don't know whether this could be done. Experience with various dosage ranges in humans I suspect is not great.

It would almost appear that there is some

individual idiosyncrasy that makes a person susceptible to having such a thing. What this means I don't know, but the low frequency with which they do, one almost has to suppose some mechanism of this type. And it's quite conceivable this is the individual that would get a neurologic reaction if he were given tetanus in the antitoxin or the rare one that occurs after typhoid, but it happens he comes in contact with pertussis first. That's all I can say.

DR. COHEN: We have one case in which the physician gave quadruple vaccine, DPT-Polio, to a baby three times, and the fourth time we interfered luckily. The first time the child had convulsion and was in shock. The second time the same. The third time the same. And then we advised to stop immediately.

But we gave diphtheria, tetanus and polio and no reaction.

DR. H. D. ANDERSON (Michigan Department of Health, Lansing and Grand Rapids, Michigan): We did a number of studies over the past six or seven years in a bunch of institutionalized mentally ill people where we were interested in recall of immunity. These people had been rested for ten to 14 years from a primary immunization that did contain a variety of multiple

antigens, including pertussis.

And then ten to 14 years later we went back to this study group and they were a huge variety of ages up to 70 years. And we started giving a dose of full half ml. booster of DPT.

I'm sorry I didn't think to bring this reprint with me, but we got a very high incidence of what we would call moderately severe reactions. There would be some systemic effects as well as local soreness and erythema.

So then we backed down to a two-tenths ml. dose, and we got a graded or a downgraded type of reaction. We still had a high percentage of what we would call moderate reaction where they complained of soreness in the arm and some evidence of febrile reaction, but at least there were less.

This I think would bear on the question of what about older groups. I mean you will expect more moderately severe reaction in a big variety of people.

There are hundreds of people in this study, and we did a parallel study at another institution, then finally decided for the purposes of our study we'd better go back to DT injections, and there we got practically no reactions in something over 1100 subjects.

DR. BRIGHAM: Dr. Wilson, we haven't heard

from Canada. Don't they have any reaction in Canada?

DR. R. J. WILSON (Connaught Medical Research Laboratories, Toronto, Canada): Dr. Chairman, I was going to ask Dr. Christensen if in his survey all of the reports of the 21 cases were actually encephalopathy with sequelae or whether many of these were just repeated convulsions.

Our experience has been the single convulsion attack has tended to be ignored. The ones where we had sequelae or death have been very few over many years, those in which there was sustained or repeated convulsive seizures and sometimes it went on and on until death. But not infrequently physicians will report convulsive seizures following high fever. These might be difficult to collect. We don't know the incidence of that.

DR. CHRISTENSEN: In the 21 children, if they had convulsions, it was specified in the questionnaire that these were recurrent things that came on at the time of the episode of encephalopathy and then continued to have recurrent convulsions subsequent to that time.

You remember I rather discounted one case where a child had a single convulsion with a febrile episode at the time of injection, because in my own experience at least this could be explainable purely on the basis of fever.

The child could get a fever from the vaccine and

then have a febrile convulsion from any cause in very young children, and I like to separate this child from the one who has a real encephalopathy, because I don't think they are the same thing at all.

But these 21, well, as we say, I think there were three that recovered completely, had no after effects after their encephalopathy episode, but the sequelae that were mentioned were continuing affairs.

DR. WILSON: The second question I would like to ask is: Have you any information on the Swedish experience where they claimed quite a high incidence of encephalopathy? Are these actually encephalopathies, do you know, or were they merely incidents recorded?

DR. CHRISTENSEN: The only thing I know is what has appeared in the British Medical Journal, which I am sure you have read as well as I. I have no personal first-hand knowledge.

DR. ELDERING: Weren't they giving a very large dose of vaccine at the time? Dr. Malmgren intimated that they were, a very heavy vaccine, which they later reduced.

DR. EMILY BANDERA (Parke, Davis and Company): I wonder if the clinical disease in the people over six years is serious enough to warrant giving the vaccine -- that is, the presently available vaccines.

DR. CHRISTENSEN: Maybe Dr. Eldering could answer

that.

DR. ELDERING: Last year -- I suppose it was unusual -- but there were two men in their forties who were hospitalized with whooping cough. And in these teenagers in high school I don't think some of them were very uncomfortably ill, but it was really quite a tragedy for some of those high school seniors in the spring when they lost six weeks out of their school year. So I think it was serious.

DR. KENDRICK: Mr. Chairman, I think there is one other factor too perhaps in addition to "was the disease serious." How much of a danger are they as a source of exposure?

DR. BANDERA: Yes.

DR. FELTON: I'd like to speak about our elder citizens. Again, they are very susceptible in families where they have a close contact, and in many of these older people they have existing lung disease. And whooping cough is a very serious thing when it occurs in an older person.

DR. CHRISTENSEN: Dr. Eldering, you related the attack rate to the interval which had occurred since the last injection.

DR. ELDERING: Yes.

DR. CHRISTENSEN: Can you relate the attack rate

to whether the individuals have had three doses of vaccine, four, or five?

DR. ELDERING: I think Dr. Lambert has that. I can dig it out for you, but I can't do it now. I have this here if you want to see me afterwards -- pages.

DR. CULBERTSON: Is there any experience on intradermal injection of older people to boost -- (inaudible)

DR. FELTON: Well, when we were working with the agglutinogen skin test we gave this material along with an intermediate material and with the sonic extract sometimes. The material as it contains more and more toxin gives us a very ugly reaction in the arm of anyone who has any difficulty. With agglutinogen nothing happens as far as the arm is concerned. You get sort of a red mark but it doesn't hurt.

The antibody titer to agglutinogen goes up in two or three days to the top of the machine. But is it protective? We don't know.

DR. BRIGHAM: Dr. Barrett, you have seen a lot of cases of whooping cough. Do you get any of these reactions of encephalopathy type things in your experience?

DR. BARRETT: I have to answer that question with great qualification, because my work is strictly in the immunizing responsibility for the Detroit Health Department.

I, unhappily for me, do not see the sick children at the hospitals, at Children's and Receiving and Herman Kiefer. But I try to keep track of the point that you're making.

And we give some 25,000 injections a year in our indigent clinics. That's some five to seven thousand children under five. And I have not heard of any encephalitis occurring out of this.

Now, they do happen once in a while at a place like Children's Hospital. They will have what they think is an immunization-related encephalopathy. But, as I say, I haven't ever gotten concerned about it, and I would like to be able to go ahead and just haven't had the time to run a controlled study and go ahead with the same material we have and go ahead with booster programs in the five to 15 year age group.

DR. WILSON: Mr. Chairman, we can't say anything about the response of adults with regard to reactions because we have closed several factories giving DPT-Polio. We don't know whether that was the diphtheria component or not, but in high school children we do. Apparently in our experience older age groups do have more severe reactions.

DR. PITTMAN: I notice that Dr. Barrett made his observations 24 hours after injection. We are dealing with

a pyrogenic organism, and usually these reactions come much earlier than 24 hours.

Dr. Bell told me that if he observed between six and eight hours his reaction rate was twice that at 24 hours. These are on the same children.

Now, in comparing two vaccines, are we going to get one that will give an early rise but a fall at 24 hours? Are we getting a true picture of the reactive rate when measuring at 24 hours?

I'd just like to make one comment, and that is I notice Dr. Barrett transposes some of the initials of the vaccine. He puts pertussis between the tetanus.

DR. BARRETT: I do that because of traditions. I resist it privately. I would readily agree that we are not measuring a true level of incidence of reactivity when we look at these children between the period of what amounts to 16 hours and 24 hours later.

This is borne out by two observations in my own work. One is that these mothers will often say they were up at night with the baby, the baby was feverish or irritable, fussy, crying all night. Yet when our nurse makes a reading of the temperature, it's within normal limits.

The second substantiation of the fact that we are not catching these babies at the peak of their reactivity --

I say "babies' advisedly; I'm talking really about children up through the age of five -- is that when we look at the same children who do have measurable febrile reactions within the 16 to 24 hour period, they are practically all gone at 48 hours. Very few persist. They are rare.

The trouble is in my situation I'd have to send my nurses out at midnight, because my clinics are held at around six to nine o'clock in the evening. This is a matter of timing and convenience as to when we can run these trials.

I do say though that relatively speaking we have an index here that we can use to measure differences between vaccines. It's just that we are not seeing them all at their peak. And I do feel that the methodology is sound.

DR. CHRISTENSEN: May I ask a question of Dr. Barrett again? I'm a pediatrician, and I distrust axillary temperatures. Would you just reassure me, please, that the correlation between axillary and rectal is good?

DR. BARRETT: Well, I have run at the clinic a number of rectals, a number of orals, and the variation between what one would consider clinically normal babies is as much as two or three degrees on normal subjects. And this is no new bit of information. I'm sure others have seen it.

I would have to say that I haven't run any extended controlled studies where the same children had an axillary and a rectal run simultaneously to see what the degree of correspondence would be.

I do say though that when I can take any group of 30 or 50 children as a sampling into the clinic for their post-primary bleeding only -- they might have had a smallpox vaccination which we wouldn't expect to cause any febrile reactivity certainly within the first 48 hours as a primary reaction -- and when one hundred per cent of these children are reported as having axillary temperatures below 98.8, which is the upper limit of my definition of normal for them, I simply offer this as an additional bit of confirmation on the reliability of the methodology of using axillary temperatures as an index if you do enough children.

On an individual 1 to 1 basis, I would still want to have additional information, a rectal temperature. But on mass trials I believe that the statistical evidence is very valid.

DR. CHRISTENSEN: I think I was just probably trying to salve my conscience for the number of times I bawled a nurse out because she took a temperature in the axilla rather than the rectum when I wanted to know what the temperature was.

DR. MURRAY: On this matter I think the point at

issue here is what Dr. Barrett is measuring is not true body temperatures. He's measuring axillary temperatures. And for his purpose this is good enough. And he's measuring them rather accurately with an instrument that has a very short time lag.

I think this is the important feature here. To think that he is measuring body temperatures I think introduces a new element into the discussion which doesn't exist.

DR. BARRETT: Thank you, Dr. Murray.

DR. BRIGHAM: Dr. Pittman, have you got anything to say about Dr. Cohen's reference here from your standpoint? Nobody seems to want to talk about it. I don't think anybody knows out here in our fields.

DR. PITTMAN: My interest has been what should be the standard of potency for therapeutic serum. That's been my particular interest. But I still have no clinical data on how much should be given to a child. This is what I would like to know.

We have a great deal of data on the therapeutic sera, and they vary per dose about, oh, 20-fold, the ones that are on the market, as far as unitage is concerned.

I wish that we had some information on what should be the potency of serum administered therapeutically.

Dr. Cohen has been trying to get some information,

but maybe he has some new information he didn't have for a while.

DR. COHEN: No, I certainly have no new information. We wanted to do a few trials at one time, and we had the serum ready in different amounts. Then pertussis disappeared in the place where we had everything ready.

So the only information I have is a casual statistic of a child who was possibly contaminated in house and was injected with serum of a known number of units and didn't get it. But I think this is not giving any more information which can be relied on.

DR. KENDRICK: Mr. Chairman, I'd be interested to know what they are giving now.

DR. BRIGHAM: Does anybody have any answers now -- what they are giving in the cases?

DR. FELTON: Everything from Robitussin to chloramphenicol.

DR. KENDRICK: I mean how much hyperimmune serum.

DR. FELTON: Well, we had a desperately ill little Indian out with us last year, and I thought she was going to die, so I called the boys at Children's Hospital at the same old station, the same stop, for serum that I could give intravenously to her. She was nine years old and I think weighed about 60 or 70 pounds.

We gave her 40 cc. reconstituted halfway, a double

concentrated, at four o'clock in the afternoon.

As I knew she would, she made a dramatic improvement over the next few hours.

We repeated this dose again next morning, and a third dose another 24 hours away. And within four days the child was clinically as well as she was before she got sick.

It took her Xray about three weeks to clear. She had myocarditis and not any cerebral complication. She was so dyspneic we thought she was going to go out.

The Philadelphia material is the only material we can use but in our hands it has been lifesaving many, many times.

DR. ELDERING: You give it intravenously?

DR. FELTON: In babies we give it to full serum tolerance, 8 cc. per pound of body weight.

DR. E. A. TIMM (Parke, Davis and Company): I'd like to ask Dr. Felton a question relative to her tissue culture system. Can you detect a cytopathogenic effect due to the pertussis by ordinary microscopy? And if so, is it a simple enough procedure so this might be applicable to a test method for neutralization?

And have you planned any studies along these lines to correlate responses?

DR. FELTON: I'm not in this work at the present

time, but we can make preparations in a fixed chamber. We have roller tubes. And any time we can pluck them out, at certain times, close them in, set them under the microscope, and take a still photograph. You see, you can follow your progressive changes in this way by doing one every 15 or 20 minutes.

I have not worked with any cell line with the pertussis. I believe this is where the gold will be if someone will be brave enough to take this up.

DR. TIMM: Will these cells grow out all right on a standard tissue culture tube?

DR. FELTON: Yes.

DR. TIMM: These are all primary plants?

DR. FELTON: Yes.

DR. PECK: I want to second Dr. Kendrick's idea that the fluorescent antibody is probably more sensitive and is picking up non-agglutinating antibody. From our experience with this modified neutralization test I think we are measuring the total amount of protective substance, whatever it is, agglutinin, opsonin, what have you.

And I'm not surprised since anFA test is more sensitive than other systems, other things too.

DR. BRIGHAM: Unless we have got one more question, we'd better stop. We've got to go to lunch.

Is there one more question?

(No response.)

If not, we'll stand adjourned.

(Whereupon, at 12:20 p.m., a recess was taken until 1:30 p.m., this date.)

AFTERNOON SESSION

(The afternoon session was convened at 1:30 p.m., Dr. Roderick Murray presiding.)

DR. MURRAY: Could we come to order, please?

I think we're setting new records for punctuality. Perhaps the luncheon was not very salubrious to conversation. Perhaps the food wasn't very good.

Before we continue with the afternoon session, could I request again that if you would let us have the slides, we will make copies of them. We haven't been able to photograph them, but for purposes of the record we can make very excellent Xerox copies which are good enough for following what has been said.

So if we could have these, we assure you that we will get them back to you before you leave.

Now, this afternoon's session deals with another aspect of pertussis vaccine problems, and this concerns attempts to evaluate the toxicity of pertussis vaccine particularly in the laboratory.

The first speaker will be Dr. Henry Piersma, who is going to speak on experiences with the pertussis vaccine toxicity test.

Henry.

DR. H. D. PIERSMA: Mr. Chairman, the quality of the pertussis vaccines used in the United States is

controlled by a number of tests performed by the manufacturer as the result of Government regulations and his own interest in making a better product.

These tests are designed to determine the potency, purity, safety and sterility of each lot of vaccine intended for use whether as such or in combination with other biological products.

Before any lot of vaccine may be released for public use, however, a protocol of the tests performed by the manufacturer, together with a sample of the lot of vaccine under consideration, must be submitted to the Division of Biologics Standards of the National Institutes of Health.

Upon review of this protocol, the Division of Biologics Standards may or may not decide to perform confirmatory tests on the lot under consideration.

If this Government agency is satisfied with a given protocol and decides that it does not wish to perform any tests on the lot submitted for release, the manufacturer is advised that the lot is approved for distribution.

However, if the agency decides to perform one or more tests on the lot under consideration, the manufacturer is advised and given an approximate date when the Government test will be completed.

With a view toward obtaining a more rapid release,

a manufacturer may request the Government to test a lot number of his product before he has completed his own tests for this product.

If the Government finds it possible to test this lot simultaneously with the manufacturer, the manufacturer must, upon completion of his own tests, submit a satisfactory protocol before approval for release can be granted.

When both the Government and a manufacturer perform tests on a given lot simultaneously, it occasionally happens that the Government test is satisfactory while the same test performed by the manufacturer is unsatisfactory. Unless the manufacturer can show that he has obtained a satisfactory result, the Government is not in a position to release that lot of product even though the Government test has been satisfactory.

The mouse toxicity test for pertussis vaccine as formerly and presently defined for use in the United States most likely has been responsible for a substantial reduction in the reaction rate encountered upon injection of this product in humans.

We wish to point out, however, that the present test is without a fixed reference point, and this weakness in the test has caused manufacturers in the United States considerable distress from time to time.

We wish to present the results of some

experimental work carried out by seven manufacturers of pertussis vaccine and discuss briefly the significance of these results.

The toxicity test for pertussis vaccine. There is general agreement among clinicians that use of pertussis vaccine is occasionally accompanied by unfavorable reactions. Certain of these reactions are severe and result in permanent damage to the central nervous system, while other reactions are comparatively mild and transitory in nature.

It has not been demonstrated that all reactions to the administration of pertussis vaccine are the result of a common cause. There is evidence, on the other hand, that some reactions are the result of an idiosyncrasy on the part of a patient, while the majority of reactions appear to be directly due to the toxicity of the vaccine injected.

Billaudelle

Recently the work of Bill-Odell and his co-workers in Sweden has resulted in some chemical characterization of the toxic components of the vaccine.

In view of the recognition by clinicians about 30 years ago that pertussis vaccine could be responsible for severe reactions, and in an effort to reduce the incidence of these reactions, the regulatory officials of the NIH felt strongly that a toxicity test in a laboratory

animal was essential.

Consequently, a toxicity test for pertussis vaccine was first introduced as a result by the Federal Government in the United States when it issued its minimum requirements for pertussis vaccine under date of March 25, 1948.

Pittman described the origin of the toxicity test as the result of studies involving the induction of skin reactions in rabbits and weight changes in mice. When a vaccine ceased to induce necrosis in a dose of 1,000 million bacteria, any weight loss induced in mice was usually regained within 72 hours.

On the basis of this correlation, a mouse toxicity test was adopted by the NIH. Pittman discarded the rabbit test because of the variability of reaction from rabbit to rabbit and because of the variability of reaction from site to site in the same rabbit.

Although the first description of this test involved a dosage of vaccine measured in terms of the total number of bacteria injected, this was subsequently revised so that now one uses the opacity unit as the unit of measurement of the dose administered to the test animal.

A recent revision of the mouse toxicity test was made officially on August 23, 1961, and Table 1 summarizes these changes. (Slide 1)

The previous regulations had no provision for testing the bulk solution of bacterial fraction. The present regulations do.

I might say these became effective on August 23, 1961.

The previous regulations tested the bulk material at ten opacity units, and the present regulations do the same for the bulk.

On the final vaccine, one tested ten per cent of the total human immunizing dose of plain vaccine, 20 per cent of total human immunizing dose of the adsorbed vaccine.

In the present regulations one tests 16.6 per cent of total human immunizing dose.

With reference to the number of mice, the previous regulations required at least five per group of the same sex. And here we are using at least ten per group.

And the requirements included the recovery of the pre-injection group weight in three days. This remains the same.

Then one simply had to exceed the pre-injection group weight in seven days. Here the group weight must average three grams per mouse at seven days.

Here we had no vaccine-related deaths permitted, and here there are five per cent.

And previously one did not have to report all tests. Now one does.

May I have the lights, please?

Following the recent changes in the toxicity test for pertussis vaccine, a committee from the Biological Section of the Pharmaceutical Manufacturers' Association, composed of several individuals representing many of the manufacturers of pertussis vaccine in the United States, decided to investigate certain aspects of the mouse toxicity test.

As the result of experience with this test over many years, members of this committee considered it possible that various strains of mice differed in their response to a given lot of vaccine. Accordingly, the committee decided to investigate this aspect of the toxicity test.

The chairman of this committee undertook to make a toxic preparation for use amongst various laboratories on a comparative test. The preparation was made from pertussis organisms grown on a charcoal ~~Ogden~~ agar/medium. After harvesting, (lactose) to 5 per cent final and merthiolate to .01 per cent final concentration were added to the suspension, which was then adjusted in density to the 100 opacity units per milliliter.

Only a small amount of the supernatant of the

original harvest suspension was discarded to permit the addition of the lactose.

Five days after the above treatment of the suspension, the suspension was incubated at 37 degrees Centigrade for 24 hours.

A sterility test at this time showed there were no viable organisms in the suspension.

The suspension was held at 2 to 5 degrees Centigrade for 27 days from harvest and then filled into vials and subsequently frozen and dried.

Distribution of this preparation was made under identification number BD-10,257.

Each of seven manufacturers were sent a supply of this toxic preparation and information for its dilution to various concentrations in terms of opacity units.

Each laboratory was asked to test this preparation at at least the $7\frac{1}{2}$ opacity unit level in at least 50 mice of the strain or strains generally employed in that laboratory for the testing of pertussis vaccine.

For each strain of mouse used in toxicity testing, each laboratory was also asked to collect data on the weight gains of at least ten mice injected with one-half milliliter of sterile physiological saline and this test run simultaneously with a testing of the toxic preparation.

All other procedures followed in performing this

toxicity test were identical to those followed in conducting the official test.

The results of this study are summarized in Table No. 2. (Slide 2)

It will be noted that the data here presented compares with the response observed in several strains of mice to the intraperitoneal injection of $7\frac{1}{2}$ units of the reference preparation under study.

From a review of this data, it is obvious that this preparation is too toxic to pass the official toxicity test for pertussis vaccine, but certain points may be drawn from this study.

First of all, it was interesting to note that the mortality rate varies from 4 to 43 per cent for various strains of mice. You can see that in this column right here. Here are the various strains of mice used, and this is the difference in mortality rate.

We are informed by statisticians that this difference in mortality rate is significant. This means that a considerable difference exists in sensitivity of various strains of mice for lethal toxin contained in pertussis vaccine.

Secondly, looking over the column indicating the weight gain in grams of the mice given one-half ml. of physiological saline only, it may be noted one strain of

mouse failed to gain an average of three grams per a seven-day observation period. This strain of mouse accordingly may not be used in testing vaccine in accordance with the official test for pertussis vaccine since animals on test must gain an average of three grams over their pre-injection weight during the seven-day test.

It is known that significant differences in the growth rate of various strains of mice exist, and each strain has its own characteristic growth curve under normal circumstances.

I think we can turn that slide off.

As mentioned previously, the toxicity test for pertussis vaccine as described officially for use in the United States most likely has served a useful purpose. Most if not all American manufacturers are satisfied that they have made a better product available commercially since the introduction of this test officially in 1948.

However, it is also true that most if not all American manufacturers of pertussis vaccine recognize that the present test has limitations which should be corrected as soon as possible.

The experimental work here presented shows that various strains of mice differ in their response to one pertussis vaccine preparation. With a spread from

4 per cent to 43 per cent in mortality response, one can state that certain strains of mice are relatively sensitive to the toxic factor or factors in pertussis vaccine, while other strains are relatively insensitive.

There are a number of practical considerations that follow as a result of this situation.

First of all, one manufacturer may select a strain of mouse for testing his product that is relatively insensitive to the pertussis toxic factor or factors, while another manufacturer may select a relatively sensitive strain of mouse for his test. If the Division of Biologics Standards tests the toxicity of vaccines from these two manufacturers and the sensitivity level of the mice used by the Government agency lies midway between that of these two manufacturers, one manufacturer's product will be released and the other's will be rejected.

Unfortunately, the sensitivity of the mouse strain used routinely by the Division of Biologics Standards to the pertussis vaccine preparation used in this study has not been determined.

However, in view of my comments in the introduction of this paper, namely, that a manufacturer may get a release on a lot of vaccine only if his results agree with those obtained by the Division of Biologics Standards, it also becomes apparent immediately that the

sensitivity level of the strains of mouse used for toxicity testing by the Division of Biologics Standards becomes the only reference point for the present official test.

It is the opinion of most of the manufacturers of pertussis vaccine that a more objective reference for evaluating the toxicity of the vaccine is needed.

In addition to the establishment of an objective reference for the toxicity of pertussis vaccine, it is also recognized that other important problems exist with respect to the use of a laboratory animal for evaluating the toxicity of pertussis vaccine.

Chief among these problems is the urgent need to relate the sensitivity level of the present mouse test to the sensitivity level of toxicity of this product when administered to humans.

In order to accomplish this, we think it imperative to recognize the differences in sensitivity levels for various strains of mice, and we hope the establishment of a reference preparation for the present pertussis toxicity test is not long delayed.

Thank you.

(Applause.)

DR. MURRAY: Dr. Piersma makes a very convincing presentation. In fact, I had difficulty keeping back the tears. (Laughter)

(Slide 1)

TABLE 1

Comparison of Previous and Present Regulations for Pertussis Mouse Toxicity Tests

Type of Preparation	Previous Regulations			Present Regulations *		
	Amount Injected	No. of Mice	Requirements for Release	Amount Injected	No. of Mice	Requirements for Release
Bulk Solution of Bacterial Fraction	No Provision			60% of Single Human Dose of Final Product	At least 10 per group	① Recover pre-injection group weight in 3 days ② Group weight must average 3.0 gms. per mouse at 7 days ③ 5% vaccine-related deaths permitted ④ All tests to be reported
Bulk Bacterial Suspension	10 Opacity Units	At least 5 per group (same sex)	① Recover pre-injection group weight in 3 days ② Exceed pre-injection group weight in 7 days ③ No vaccine-related deaths permitted	10 Opacity Units		
Final Vaccine	10% of Total Human Immunizing Dose (Plain Vaccine) 20% of Total Human Immunizing Dose (Adsorbed Vaccine)			16.6% of Total Human Immunizing Dose		

* Effective Aug. 23, 1961

TABLE 2 (Slide 2)

Results of Toxicity Tests on Dried Reference No. BD-10257 from Seven Manufacturers

Manufacturer	Dose in Opacity Units	Strain of Mouse	Deaths/Total	Per Cent Mortality	Saline Controls 7-day Weight Gains
A	7.5	Carworth Females	2/50	4	—
E	"	CFW Males	2/50	4	4.0
C	"	CF1 Males	3/60	5	3.5
B	"	McAllister Males	13/150	9	—
F	"	CFW Females	6/50	12	2.2
B	"	Cox Males & Females	6/50	12	—
D	"	?	7/50	14	—
C	"	CF1 Females	2/10	20	3.2
C	"	C Males	12/60	20	9.2
C	"	C Females	13/60	22	6.0
F	"	Lab. Supply	13/50	26	4.8
G	"	Dierolf Females	29/100	29	3.5
A	"	Taconic Females	21/50	42	—
C	"	Detwiler Males	13/30	43	4.3

But we'll hear more on this.

The next is from Mr. Donald Marshall on his experiences -- at least, experiences in industry -- with the freedom-from-toxicity test.

Mr. Marshall.

MR. DONALD E. MARSHALL: Well, what I'm going to report may be a bit redundant. I didn't hear Henry's paper. But by the time you have listened to mine I'm sure Dr. Murray may be borrowing handkerchiefs. (Laughter)

Since the inception of the freedom-of-toxicity test in 1952, the minimum requirements for pertussis vaccine, manufacturers have, of course, accumulated a large mass of test data. To attempt to summarize these would be a herculean task, and there would be really little interest in simply reviewing them.

The modern production of biological products in volume demands careful integration of planning, production, control testing, finishing, and inventory control operations.

It is only natural, therefore, that manufacturers should focus their attention upon any test which adversely affects the smooth flow of this sequence of operations.

In discussions among representatives of producers of pertussis vaccine, it was obvious soon after its inception that the freedom-of-toxicity test was a frequent

offender in production delays.

It was also soon obvious that the problems were not only attributable to the mouse toxic factors of B. pertussis but to the test itself.

These problems have been multiplied manyfold with the advent of the requirement in 1961 of a satisfactory test for freedom-of-toxicity on bulk suspensions prior to their incorporation into final products.

To illustrate some of these practical problems, I have asked several major producers to give me examples of their experiences with this test. I have summarized some of these along with examples from my own company, and copies of these have been distributed I believe for your inspection.

I won't take time to go over these in detail, but the first three pages summarize data from products prepared from suspensions which have passed the freedom-of-toxicity test yet have failed the test in the final form.

The last page has a few cases apparently made before the latest requirements of products prepared from suspensions which failed the freedom-of-toxicity test but which gave final products which passed the test.

In dealing with suspensions or final products, especially those which were borderline with respect to passing this test, it was soon apparent that this test was

not very precise. To determine how precise, we have tested four bulk suspensions of B. pertussis having various degrees of toxicity at a dose of ten opacity units per half cc. dose given intraperitoneally.

This was done in groups of 50 mice which were individually weighed at 0, 3 and 7 days post-injection. These were weighed within a tenth of a gram.

A control group of 50 similar mice were not injected and were weighed as the others.

These data have been analyzed by our statisticians, and the mean weight gains plus or minus two standard deviations have been calculated.

Could I have the first slide, please?

You note the decreasing degree of toxicity from the top down from these four suspensions, and you will notice the rather fast growing mouse as exhibited by the weight gain of eight grams in seven days.

Obviously, with a mean weight gain here of a half a gram and two standard deviations greater than that, the test is not too precise.

The rather poor precision of the test can be graphically described in the next slide in which we have taken these data and the statisticians have assumed a vaccine with a true weight gain of 3.0 grams, which is the standard required to pass, and have calculated from the

(Slide 2)

preceding data the number of mice which are needed to insure with 95 per cent confidence that the true weight gain is different than the assumed 3.0 standard.

Obviously, with a small difference you would have to use a tremendous number of mice. And as you get down into these areas here you notice, for instance, at 3.4 grams you would need 73 mice to pass this 19 out of 20 times.

And the important point is that with the standard ten mouse test, a true weight gain of 4.1 grams would have to be present in your vaccine to pass this 19 out of 20 times.

I think we can take the slide off.

It is generally accepted that different strains of mice vary in susceptibility to the toxins of B. pertussis. Further, many factors, some subtle, may govern the weight gain of mice.

We have found it to be true that mice of the same strain, fed the same diet but held in different environments within our own company, will show different results in the freedom-from-toxicity test.

Diet is obviously important. One manufacturer reports that the addition of dried bread cubes to the normal ration in the first, second or third day has increased weight gains as much as 30 to 50 per cent.

These are simply a few examples of the factors

other than the product itself which may affect this test.

Producers are aware that their tests are subject to check by the Division of Biologics Standards.

In lieu of a reference preparation, therefore, it is obvious that the ultimate standard for passing this test is the strain of mouse and its method of feeding and handling in the Government controlled laboratory.

To illustrate the difference which may occur between laboratories, I have summarized the results of some comparative test results between our laboratory and the Division of Biologics Standards.

In the summer of 1962 we were having difficulty in obtaining passing freedom-of-toxicity tests on our bulk suspensions. The DBS graciously agreed to test four lots of production material which we had been unable to pass in our freedom-of-toxicity tests. The results of these tests are summarized in the next slide. (Slide 3)

These four suspensions which we had been unable to pass, some of which were phemerol preserved and I believe one was merthiolate preserved, were tested in this number of mice which gave the average seven-day weight gain here. Obviously these failed the test.

The dose here was ten opacity units, incidentally. The results obtained at the DBS on similar ten opacity unit tests averaged in these columns passed these suspensions.

These data show that the mouse used at the DBS or its methods of feeding and handling there gave greater weight gains than those obtained in our laboratories.

Accordingly, we set out to find the strain of mouse which would compare in susceptibility in our laboratory with the results obtained on these same suspensions used at the DBS.

The results of tests on two of these suspensions in different strains are summarized in the next two slides. Could I have the next one, please? (Slide 4)

Here's the suspension cited on the previous slide with the DBS weight gain of 4.2 average. These are mouse strains, sources, on test dates, all given the same dose, with the average seven-day weight gain given summarized here and mortality in this column.

The strains which gave the best weight gains, of course, were the Rawley, Spartan and the Rawley, Spartan repeated in this particular test.

These are fairly comparable to what was obtained at DBS on this same suspension.

Could I have the next one, please? (Slide 5)

One of the other suspensions which gave an ideal almost weight gain of 3.1, to just pass the test, tested in again these different strains that we used previously, same dose and everything, with mortality rates here, pointed out

that the Spartan here and here averaged closest of any of these strains to the DBS results.

We can take the slide off, please.

The superior results judged as comparing to the DBS results obtained with the Spartan mouse led us to adopt this animal for routine testing. Unfortunately, within a few weeks, an apparent abrupt change occurred in this mouse and it became no better than other strains we had previously tried. The supplier denied any change in the breeding of this strain.

We checked it back with these same suspensions.

Fortunately, another strain was found which has given results comparable to those obtained at the DBS. However, now it appears recently that this mouse is changing, and we may soon be in trouble again.

The point to be emphasized here is that in order to prevent undue disruptions in production one must arbitrarily select a strain of mouse for this test which gives the reaction to the toxic factors of B. pertussis similar to those mice used at the DBS.
This again we believe points to the need of some sort of a reference preparation for controlling the animal variation factor.

To summarize the feeling in industry may be presumptive on my part. However, in discussing this

problem among industry representatives, one soon learns that the test is not held in high regard as to its precision and is questioned as to its significance in controlling pertussis vaccine-containing products.

We have shown that the test results are dependent upon the strain of mouse used or the method of handling them.

We have presented evidence that suspensions passing this test may yield final products which fail the test.

Accordingly, since only final products are administered, many question the need for a test on bulk suspensions prior to incorporating them in final products.

Finally, parent suspensions or even final products must sometimes be treated in various ways such as incubating at 37 degrees, as is shown in these pass-outs, to pass this test. Some of this treatment undoubtedly destroys antigen, which means that higher opacity units must be included in the final product to pass the potency test.

Thus, the recipient of the product will be given more bacterial protein than otherwise would have been administered. This may induce greater reactions.

The end result of this cycle appears to be diametrically opposed to the rationale for having a

freedom-from-toxicity test.

As a result of our experience with the freedom-of-toxicity tests, we hope -- we in industry I should say hope -- that the question of the necessity of testing bulk suspensions may be reconsidered and that a method for controlling the variations of the test between laboratories, such as a reference preparation might provide, may soon be developed.

Thank you.

(Applause)

DR. MURRAY: Thank you very much. I hope that when we come to the discussion period that some of these thoughts expressed by Mr. Marshall and also by Dr. Piersma may be discussed in greater detail.

I think there is an area here that should be discussed.

I won't prejudice this discussion by making any remarks at this time except to say that there's no tears this time. It's just an admiration for your heroic fortitude in the face of a perverse fate. (Laughter)

The next item on the agenda -- and we're going along very nicely I'll say -- is by Miss Frances Angela, on the relationship of the concentration of adjuvant to the toxicity in mice in products containing pertussis vaccine.

MISS FRANCES ANGELA: I'd like to amend the title

(Slide 1)

B. PERTUSSIS FREEDOM-FROM-TOXICITY TEST
MEAN WEIGHT GAINS AND 95% CONFIDENCE INTERVALS

LOT. NO.	MEAN WEIGHT GAIN ($\pm 2S_d$) GM. (d 3 DAYS	(d 7 DAYS
096671	0.2 (± 0.30)	0.5 (± 0.58)
096668	1.7 (± 0.36)	2.4 (± 0.50)
099397	3.2 (± 0.38)	5.7 (± 0.72)
098244	3.7 (± 0.40)	5.6 (± 0.70)
UNINOCULATED	4.6 (± 0.24)	8.2 (± 0.44)

(Slide 2)

B. PERTUSSIS FREEDOM-FROM-TOXICITY TEST

"TRUE" WT. GAIN OF MICE	MICE NEEDED TO INSURE (WITH 95% CONFIDENCE) THAT THE TRUE WT. GAIN IS DIFFERENT THAN 3.0 GM.
1.9	10
2.3	24
2.6	73
2.7	128
2.8	289
2.9	1,156
"3.0"	-
3.1	1,156
3.2	289
3.3	128
3.4	73
3.7	24
4.1	10

(Slide 3)

MANUFACTURER'S TEST RESULTS VS. D.B.S. TEST RESULTS

SUSPENSION NUMBER	MANUFACTURER'S TESTS NO. MICE	MANUFACTURER'S TESTS AVG. 7 DAY WT. GAIN (GM.)	D.B.S. TESTS NO. MICE	D.B.S. TESTS AVG. 7 DAY WT. GAIN (GM.)
090369	40	2.0	20	3.3
093295	40	2.5	20	4.2
093298	20	1.4	40	3.1
091411	20	1.2	20	4.0

(Slide 4)

EFFECT OF MOUSE STRAIN

SUSPENSION NUMBER	MOUSE STRAIN	DATE	AVG. 7 DAY WT. GAIN (GM.)	% MORTALI
093295	STOUT	7-17-62	2.6	20
	LAB SUPPLY	8-01-62	3.0	0
	RAWLEY	8-03-62	3.7	10
	SPARTAN	8-03-62	3.3	0
NOTE: D.B.S. 7 DAY AVG. WT. GAIN WAS +4.2 GM.	CF-1	8-17-62	1.6	0
	RAWLEY	8-17-62	4.8	15
	SPARTAN	8-17-62	4.5	0
	CFW	8-17-62	3.6	0

(Slide 5)

EFFECT OF MOUSE STRAIN

SUSPENSION NUMBER	MOUSE STRAIN	DATE	AVG. 7 DAY WT. GAIN (GM.)	% MORTALITY
093298	STOUT	7-17-62	+1.4	20
	LAB SUPPLY	8-01-62	+1.3	0
NOTE: D.B.S.	RAWLEY	8-03-62	+1.5	0
7 DAY AVG. WT. GAIN = 3.1 GM.	SPARTAN	8-03-62	+2.5	0
	CF-1	8-17-62	+1.5	0
	RAWLEY	8-17-62	+1.7	10
	SPARTAN	8-17-62	+4.5	0
	CFW	8-17-62	+2.2	10

Examples of "Toxic" D.T.P. final products prepared from suspensions which passed the freedom-of-toxicity test

Pertussis Component	7 Day wt. gain (gm.)	Mortality	Conclusion
#1	+4.6	0	passes
#2	+4.0	2.5%	passes
D.T.P. Lot A	+3.8	10.0%	fails
#1	4.6	0	passes
#2	4.0	2.5%	passes
D.T.P. Lot B	2.6	20.0%	fails
#1	+4.6	0	passes
#2	+4.0	2.5%	passes
#3	+4.0	2.5%	passes
D.T.P. Lot C	+2.2	10.0%	fails
Lot 101 (pool)	no data available		passed (1 test)
Lot 25			passed (2 tests)
Lot 26			passed (4 tests)
Lot 27			passed (4 tests)
Lot 28			passed (5 tests)
D.T.P. Lot D			passed (3 tests)
Lot 670	0.8	0	fails (11-12-62)
Lot 670 (heated @ 37°C.)	0.8	0	fails (11-19-62)
Lot 670 (" " ")	4.8	0	passes (11-30-62)
Lot 670 (" " ")	3.2	0	" "
Lot 670 (2nd heating)	3.5	0	passes (12-21-62)
D.T.P. Lot 358	0.57	10.0%	fails (12-14-62)
" " "	-0.6	50.0%	" "
D.T.P. Lot 358 (heated @37°)	4.7	0	passes (12-24-62)
" " " "	4.5	0	" "
Lot 853	1.5	10.0%	fails (4-16-63)
Lot 853 (Heated @ 37°C.)	3.2	0	passes (5-3-63)
P $AlPO_4$ Lot 020	4.8	20.0%	fails (8-20-63)
" " " "	2.2	0	" "
P $AlPO_4$ Lot 020	1.2	20.0%	fails (8-30-63)
" " " "	2.3	0	" "
P $AlPO_4$ Lot 020 (heated @37°C.)	4.05	0	passes (9-17-63)
" " " " "	2.7	10.0%	" "
P $AlPO_4$ Lot 020 (Heated @37°C.)	4.5	0	fails (9-20-63)
" " " " "	-0.75	20.0%	" "
P $AlPO_4$ Lot 020	-0.3	10.0%	fails (10-8-63)
" " " "	1.75	0	" "
" " " "	1.35	0	" "
" " " "	2.46	10.0%	" "

Pertussis Component	7 Day wt gain (gm.)	Mortality	Conclusion
Lot 105	4.6	0	passes (1-5-62)
" "	5.0	0	" "
Lot 410	[illegible]	0	[illegible]
" "	[illegible]	[illegible]	" "
Lot 410 (heated @37°C.)	[illegible]	[illegible]	fails (6-25-62)
" " " "	[illegible]	[illegible]	" "
Lot 410 (2nd heating)	[illegible]	[illegible]	passes (7-20-62)
" " " "	[illegible]	[illegible]	" "
Lot 412	[illegible]	[illegible]	fails (6-18-62)
" "	[illegible]	[illegible]	" "
Lot 412 (heated @37°C.)	[illegible]	[illegible]	fails (7-3-62)
" " " "	2.5	0	" "
Lot 412 (2nd heating)	[illegible]	0	fails (7-20-62)
" " " "	[illegible]	10.0%	" "
Lot 412 (2nd heating)	[illegible]	0	fails (7-31-62)
" " " "	[illegible]	10.0%	" "
Lot 412 (3rd heating)	[illegible]	0	fails (8-14-62)
" " " "	[illegible]	0	" "
Lot 412 (3rd heating) (Spartan Mice)	[illegible]	0	passes (8-24-62)
D.T.P. Lot 403	3.2	0	fails (9-24-62)
" " "	2.8	30.0%	" "
" " "	1.6	0	fails (9-28-62)
" " "	1.3	0	" "
D.T.P. Lot 403 (heated @37°C.)	1.6	20.0%	fails (10-10-62)
" " " " "	0.8	10.0%	" "
" " " " "	3.5	0	fails (10-12-62)
" " " " "	2.4	10.0%	" "
" " " " "	[illegible]	10.0%	fails (10-19-62)
" " " " "	[illegible]	[illegible]	" "
" " " " "	[illegible]	[illegible]	" "
" " " " "	[illegible]	[illegible]	" "
D.T.P. Lot 403 (2nd heating)	3.4	0	passes (11-2-62)
" " " " "	3.2	0	" "

Pertussis Component	7 Day wt. gain (gm.)	Mortality	Conclusion
Lot 240	3.9	10.0%	passes (1-3-63)
" "	3.1	0	" "
Lot 242	4.9	0	passes (1-25-63)
" "	3.4	0	" "
D.T.P. Lot 465	0.7	10.0%	fails (4-24-63)
" " "	0.5	0	" "
D.T.P. Lot 465 (heated @37°C.)	0.4	10.0%	fails (5-6-63)
" " " " "	1.2	10.0%	" "
D.T.P. Lot 465 (2nd heating)	2.2	0	fails (5-22-63)
" " " " "	4.0	20.0%	" "
			Diluted approximate 18% and transferred Lot 665.
D.T.P. Lot 665	1.6	10.0%	fails (7-12-63)
" " "	0.7	10.0%	" "
D.T.P. Lot 665 (heated @37°C.)	5.0	0	passes (8-13-63)
" " " " "	2.95	0	" "
Lot 361	1.8	0	fails (10-12-62)
Lot 361	2.3	0	fails (10-19-62)
Lot 361 (heated @37°C.)	-0.6	0	fails (11-12-62)
" " " "	1.7	0	" "
Lot 361 (2nd heating)	0.6	[illegible]	fails (11-19-62)
Lot 361 (2nd heating)	3.9	[illegible]	passes (12-17-62)
D.T.P. Lot 322	1.0	10.0%	fails (12-20-62)
" " "	-2.0	30.0%	" "
" " "	2.0	0	fails (12-20-62)
" " "	3.0	10.0%	" "
D.T.P. Lot 322 (heated @37°C.)	2.6	20.0%	fails (1-2-63)
" " " " "	3.9	0	" "
" " " " "	2.2	10.0%	fails (1-2-63)
" " " " "	2.0	50.0%	" "
D.T.P. Lot 322 (2nd heating)	2.5	10.0%	fails (1-23-63)
" " " " "	3.4	10.0%	" "
Retest:			
D.T.P. Lot 322 (2nd heating)	3.4	0	passes (2-1-63)
" " " " "	3.7	0	" "

Examples of "Non-Toxic" D.T.P. final products prepared from suspensions which failed the freedom-of-toxicity test

Pertussis Component	7 Day wt. gain (gm.)	Mortality	Conclusion
#1	+5.4	30%	fails
#2	+1.7	15%	fails
D.T.P. Lot A	+3.5	0	passes
#1	+5.4	30%	fails
#2	+1.7	15%	fails
#3	+1.6	45%	fails
D.T.P. Lot B	+4.0	5%	passes
#1	+5.3	30%	fails
#3	+1.6	45%	fails
#4	+2.3	5%	fails
D.T.P. Lot C	3.9	0	passes
#1	+5.3	30%	fails
#4	+2.3	5%	fails
#5	+1.2	70%	fails
D.T.P. Lot D	+3.9	0	passes

sufficiently to assure you that we don't have mice in our products. (Laughter)

And I don't have whooping cough. (Laughter)

I think we're all singing the same tune apparently, because when we looked over the results of our toxicity test we couldn't entirely dismiss the suspicion we were testing the mice rather than the products.

We have always, since the toxicity test started, included uninjected controls. But because of all the puzzling variations that occurred in our testing and re-testing at various stages of manufacture, in 1962 we began to include another set. This consisted again of the uninjected controls and one set that received half a ml. of saline and usually another that received plain aluminum phosphate. One of these might be omitted depending on what we were testing.

The amount which the mice received was two-tenths mil of aluminum. I'm expressing this as aluminum just to transfer results later on.

All of the mice were from our colony, the Michigan Department of Health colony of Webster mice. And in general there were ten mice per group. Ordinarily these were housed 20 mice per pan. Occasionally there were only ten mice per pan. And there seemed to be no appreciable difference in the weight gain.

Some of this is summarized in the first table that we gave you. You will notice there were an appreciable number of deaths occurring in the control group. Of the four deaths that occurred in the uninjected mice, two of them occurred in one test.

The greatest range of weight gains as well as the greatest number of tests in which mice gained less than three grams were among the females but the mean of the weight gains were essentially the same.

At one time we had an impression that maybe you could pass it if you started with smaller mice, but that didn't work out when we looked them over.

When all of this was tabulated, there didn't seem to be any difference according to the initial weight of the mice whether it was under or over 15 grams.

In all of this it was the same lot of aluminum phosphate that had been used.

In the second table we tested seven different lots, and one was eight years old and one was two months old, and there doesn't seem to be any great difference in the weight gains in any of those. The ones that showed less than three grams were repeated and they showed adequate gains on that.

In Table 3 there is an illustration of the effect on the toxicity test of various concentrations of aluminum

phosphate with and without pertussis added.

The effect of the increased concentration of aluminum phosphate in combination with uniform amounts of pertussis is quite distinct, but I don't really know what it means.

The addition of a non-toxic pertussis vaccine results in products which by the standards of the test are all toxic. Does this mean that the aluminum phosphate has potentiated a latent toxicity of the vaccine, or does the test represent a protest by the mice against a double dose of adjuvant, the aluminum phosphate and the pertussis?

We made additional preparations from two batches of pertussis. Lot 367 was a pool on which toxicity tests had been satisfactory. That is, the bulk saline tests had been satisfactory. The other one, Lot 1551, was a recently harvested single strain known tobe toxic. Each lot was combined with aluminum phosphate and with aluminum hydroxide. The adjuvants were diluted to contain equivalent amounts of aluminum per dose.

The four tests made within two weeks are summarized in very complicated Table 4. In this table you will see an illustration of the results that are rather baffling.

At the bottom of the table, opposite "Controls," you will notice tests 1, 2 and 3 gave satisfactory results with Lot 367, but test No. 4 raises a doubt.

All the tests on Lot 1551 diluted in saline are clearly unacceptable.

Above this, in the aluminum hydroxide series, in test 3 with aluminum hydroxide alone there is one mouse in each of the three lower levels of aluminum concentration that died, although the weight gains are quite adequate.

It makes you wonder which is more important, death or weight gain.

This is the sort of result that occurs in pertussis testing and leads one to test in more and more mice hoping for assurance of the safety of the product. Unfortunately, additional tests sometimes add to the confusion.

Lot 367 in combination with aluminum hydroxide seems acceptable. But the frankly toxic Lot 1551 narrowly misses being acceptable when combined with aluminum hydroxide. So that it makes you wonder whether this is detoxifying pertussis or concealing toxicity.

At the top of the table, in the aluminum phosphate series, all groups which received aluminum phosphate alone gained well and no deaths occurred. In combination with Lot 367 toxicity in terms of the standard of the test is diminished with decreasing amounts of adjuvant. With Lot 1551 the toxic appearance is intensified at all levels of

adjuvant concentration compared with the saline-diluted or aluminum hydroxide-adsorbed preparations.

All of these preparations in Table 4 were concerned with pertussis that had been phemerol-preserved and with relatively recent origin. We wanted to include tests on a definitely non-toxic lot so we selected Lot 313. This was a pool of several strains harvested ten years ago and stored at 5 degrees in the presence of two-hundredths per cent merthiolate. We have routinely used bulk suspensions stored for three to five years to prepare DTP vaccines for distribution. Based on the absence of complaints, we believe this product to be highly acceptable in the field.

Tested in 1956 in five mice with 12½ opacity units per dose, this bulk suspension gave satisfactory results.

Preparations were made with aluminum phosphate and aluminum hydroxide as previously described. The results are shown in Table 4-A, and they are rather surprising.

Saline-diluted suspension was satisfactory. The aluminum phosphate-adsorbed material showed deaths in all groups. And the aluminum hydroxide had one death at the highest aluminum concentration.

Note also that the mice injected with saline

gained less weight than those injected with saline plus pertussis.

These results raise some doubts regarding the relationship between the toxicity test in mice and the unfavorable reaction.

I am going to make a statement that is going to get my neck cut off by somebody according to the previous statements. It's our practice and probably of most manufacturers to test pertussis suspension before use diluted in saline and also adsorbed on aluminum phosphate. In our records there are instances of the saline-diluted material giving poorer results than the aluminum phosphate-adsorbed. In general, however, to inject the suspension adsorbed on aluminum phosphate seems to be a more stringent test.

It would seem logical to include in the requirements a test of a sample of the bulk suspension in combination with the amount and kind of adjuvant intended for use in the final product.

Shall I crawl under the table now?

Perhaps consideration might also be given to decreasing the maximum amount of the adjuvant permitted in products containing pertussis.

The present allowable amount of 20 milligrams of alum is equivalent to approximately 1.1 milligrams of

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TABLE I

WEIGHT INCREASE IN TOXICITY CONTROL MICE

	MALES			FEMALES		
	0.2 mg. Al per 0.5 ml. $AlPO_4$	Not injected	0.5 ml. saline	0.2 mg. Al per 0.5 ml. $AlPO_4$	Not injected	0.5 ml. saline
No. of deaths/No. of tests	2/24	4/24	1/22	2/39	3/39	2/38
No. of tests with avg. weight increase = 3 gm. or less	0	0	1	4	2	1
Range of avg. weight increase (grams)	3.5 to 7.2	3.5 to 8.0	3.0 to 7.4	2.0 to 6.8	1.6 to 6.6	2.7 to 6.5
Mean weight increase (grams)	5.1	5.5	4.6	4.2	4.4	4.3
No. of tests in which avg. weight increase						
in uninjected mice						
was > in saline-injd. mice		10			20	
was = to saline-injd. mice		0			1	
was < in saline-injd. mice		11			13	
in uninjected mice						
was > in $AlPO_4$-injd. mice		13			22	
was = in $AlPO_4$-injd. mice		0			1	
was < in $AlPO_4$-injd. mice		9			12	
in saline-injd. mice						
was > in $AlPO_4$-injd. mice		12			17	
was = to $AlPO_4$-injd. mice		1			2	
was < in $AlPO_4$-injd. mice		7			17	

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TABLE II

AVERAGE WEIGHT GAINS IN MICE
INJECTED WITH VARIOUS LOTS OF $AlPO_4$

Injn. = 0.2 mg. Al (±0.006 mg.) per 0.5 ml. $AlPO_4$

$AlPO_4$		11-23-62 Female		12-21-62 Female		12-21-62 Male	
Lot No.	Date Prepared	I	II	I	II	I	II
10	7-23-54	+ 3.6 gm.	+ 3.4 gm.				
15	10-22-54	+ 2.7 gm.	+ 3.5 gm.	+ 5.2 gm.	+ 5.1 gm.	+ 5.6 gm.	+ 6.3 gm.
49D	4-23-58	+ 2.6 gm.	+ 3.0 gm.	+ 5.7 gm.	+ 4.8 gm.	+ 4.4 gm.	+ 5.1 gm.
Q53	11-4-60	+ 4.9 gm.	+ 3.8 gm.				
53H	11-4-60	+ 4.0 gm.	+ 3.0 gm.				
60	2-2-62	+ 4.4 gm.	+ 4.3 gm.				
63	9-20-62	+ 4.0 gm.	+ 4.2 gm.				
Not injected			+ 4.7 gm.		+ 5.2 gm.		+ 6.2 gm.
0.5 ml. Saline					+ 5.2 gm.		+ 5.7 gm.

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TABLE III

AVERAGE WEIGHT GAINS IN MICE
INJECTED WITH $AlPO_4$ OR $AlPO_4$ + PERTUSSIS VACCINE

$AlPO_4$		Avg. increase/	$AlPO_4$ + Pertussis		Avg. increase/
Lot No.	mg. Al./0.5 ml. dose	No. of S.	Lot No.	o.u./dose	No. of S.
53	0.8 mg.	4.5 gm./10	Q365	8 o.u.	0.9 gm./8
	0.4 mg.	2.9 gm./10	"	"	1.9 gm./9
	0.2 mg.	4.9 gm./10	"	"	2.6 gm./10
	0.1 mg.	4.4 gm./9	"	"	3.3 gm./9
60	0.8 mg.	4.1 gm./10	Q365	8 o.u.	1.1 gm./8
	0.4 mg.	3.5 gm./10	"	"	0.9 gm./10
	0.2 mg.	3.8 gm./10	"	"	1.7 gm./10
	0.1 mg.	2.8 gm./10	"	"	2.6 gm./10
63	0.8 mg.	4.4 gm./10	Q365	8 o.u.	0.7 gm./8
	0.4 mg.	2.6 gm./10	"	"	1.9 gm./10
	0.2 mg.	4.6 gm./10	"	"	2.8 gm./10
	0.1 mg.	5.3 gm./10	"	"	4.8 gm./8
			Saline + Q365	8 o.u.	3.6 gm./10
			Saline only	0	3.4 gm./10
			Not injected	0	3.5 gm./10
			Not injected	0	4.8 gm./10

All tests done with Female mice.
Initial weight range per group = 140 - 156 gm.

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TABLE IV

AVERAGE WEIGHT INCREASE(+) OR DECREASE(-) IN MICE/NUMBER OF SURVIVORS
(all survived unless otherwise shown)

mg. Al per dose	$AlPO_4$ 5 M 5 F Test 1	$AlPO_4$ 5 M 5 F Test 2	$AlPO_4$ 5 M 5 M Test 3	$AlPO_4$ 8 M 6 F Test 4	$AlPO_4$ + Pert.#367=10 o.u./dose 5 M 5 F Test 1	5 M 5 F Test 2	5 M 5 M Test 3	8 M 6 F Test 4	$AlPO_4$ + Pert.#1551=10 o.u./dose 5 M 5 F Test 1	5 M 5 F Test 2	5 M 5 M Test 3	8 M 6 F Test 4
0.8	+4.0 +4.2	+7.2 +5.6	+3.0 +4.0	+4.6 +3.0	+2.3/4 +3.8	+0.6/4 +3.3/3	+2.0 +3.8	+1.5 +2.7/4	-2.2/2 +1.2/4	+2.9/4 0/0	+3.9/3 +0.1/3	0/4 +0.9
0.4	+4.4 +4.2	+7.2 +5.4	+4.6 +4.2	+5.6 +3.8	+1.6 +2.8	+3.5/4 +4.4	+4.1/4 +3.6	+3.0 +2.5	-0.7/3 +1.7/4	+2.0 +3.0	+2.7/3 0/4	+1.4 +3.2/5
0.2	+4.8 +4.2	+6.6 +5.2	+4.0 +4.6	+4.7 +3.9	+4.0 +5.0	+5.2 +6.6	+0.7/4 +2.4	+5.2 +1.6	+1.4 +1.9	+1.0 +3.2/4	+2.9/4 +1.8/3	+1.0/7 +1.3
0.1	+6.2 +4.2	+6.6 +4.6	+3.6 +4.6	+4.6 +5.2	+4.0 +5.0	+7.2 +6.2	+4.0 +3.0	+3.6 +5.2	+1.4 +1.6	+2.0 +2.4	+3.0/4 +2.2	-0.8 +3.3
	$Al(OH)_3$				$Al(OH)_3$ + Pert.#367=10 o.u./dose				$Al(OH)_3$ + Pert.#1551=10 o.u./dose			
0.8	+4.8 +4.4	+7.4 +4.6	+5.0 +5.2	+4.0 +6.3	+4.4 +4.6	+6.6 +3.6	+2.8 +4.4	+5.1 +4.5	+2.0 +3.6	+3.2 +2.2	+0.7/4 +2.0	+1.8 +2.5
0.4	+7.8 +3.6	+6.6 +5.8	+5.0 +6.0/4	+4.6 +5.7	+5.4 +4.6	+5.4 +3.0	+3.2 +4.6	+5.5 +5.2	+2.0 +2.8	+4.0 +2.8	+2.6/4 +4.0/4	+2.8 +2.5
0.2	+5.8 +4.2	+5.4 +4.2	+5.0 +7.4/4	+4.5 +5.3	+5.2 +4.2	+6.2 +5.4	+4.0 +4.2	+4.4 +3.3	+1.6 +2.2	+2.8 +3.2	+2.0 +2.0	+0.4 +3.0
0.1	+5.4 +4.8	+7.4 +4.2	+3.0/4 +4.6	+4.5 +5.5	+3.3/4 +3.2	+6.1 +5.6	+2.6 +4.2	+4.5 +5.2	+2.8 +1.3/4	+6.3/4 +2.4	+2.8 +2.2	+0.1 +3.3
	0.5 ml. Saline				Saline + Pert.#367=10 o.u./dose				Saline + Pert.#1551=10 o.u./dose			
Controls:	+6.6 +5.2	+7.2 +5.8	+5.0 +3.8	+4.1 +4.0	+2.0 +3.6	+6.6 +4.4	+3.4 +2.8	+2.5/6 +2.8	+1.1/3 +0.9/4	+2.2 +1.8	+1.2 +0.4/4	+2.5 +2.2
	+5.0 +4.4	+7.8 +5.6			+4.6 +3.4	+4.4 +5.4			+4.0/4 +1.9/4	+1.6/4 +2.0		
	Not injected											
	+5.6 +3.8	+7.8 +5.8	+5.6 +5.4	+4.5 +5.2								

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TABLE IV-A

(+) (-)

AVERAGE WEIGHT INCREASE OR DECREASE IN MICE/NUMBER OF SURVIVORS
(all survived unless otherwise shown)

Toxicity Tests on Pertussis Harvested in 1953
and Preserved with 0.02% Merthiolate

mg. Al per dose	$AlPO_4$ 5 M 5 F	$AlPO_4$ + Pert.#313=10 o.u./dose 5 M 5 F
0.8	+6.8/4	+2.5/3
	+6.0	-0.6/2
0.4	+7.2	+4.5/4
	+5.2	+1.2/4
0.2	+8.2	+1.2
	+5.8	+2.4/4
0.1	+8.2	+5.0
	+6.4	+3.7/3
	$Al(OH)_3$	$Al(OH)_3$ + Pert.#313=10 o.u./dose
0.8	+6.8	+3.2/4
	+5.4	+2.8
0.4	+7.0	+3.4
	+3.2	+3.4
0.2	+5.6	+5.2
	+4.8	+4.6
0.1	+6.4	+5.4
	+4.8	+4.0
	0.5 ml. Saline	Saline + Pert.#313=10 o.u./dose
Controls:	+3.8	+5.0
	+2.8	+5.0
	Not injected	
	+6.0	
	+5.2	

aluminum, which is a quantity greater than any that we used in these particular experiments.

Thank you.

(Applause)

DR. MURRAY: Thank you very much.

It looks like an interesting session. (Laughter)

Now Dr. Pittman will present data on the testing of toxicity. Oh, Mrs. Cox is going to. Pardon me. The paper is by Dr. Pittman and Mrs. Claire Cox, and it will be presented by Mrs. Claire Cox -- 9.5 years of toxicity testing. It's a very accurate period of time.

MRS. CLAIRE B. COX: May I preface my remarks by saying that we are not unhappy with our mice. We think we are testing product, not mice, at this point.

A brief review of the results of the freedom-from toxicity testing carried out by the Division of Biologics Standards during the past 9.5 years will be presented. The data are of particular interest in that a single strain of mice of one sex has been used throughout this period, thereby providing a basis of comparison of toxicity not only between the products of different manufacturers but of consistency within a single product.

I had a fairly long, detailed explanation of the test, but I think that you all are fully acquainted with the development of the test now so I will delete that.

I will now go to the routine testing. Following the revision of the test in 1953, and concurrently with the beginning of the use of aluminum phosphate as an adjuvant, some lots of a few manufacturers failed to pass the toxicity test. To find the cause, 280 lots of vaccine submitted to the Division during an 18-month period were tested for toxicity.

This study showed that vaccines containing aluminum phosphate were more toxic than those containing alum or aluminum hydroxide and that toxicity for a single manufacturer's product was related to the amount of aluminum phosphate present, but not necessarily between manufacturers' products. One manufacturer's product might contain almost twice as much as another's product and yet not be more toxic. The difficulty was resolved by reducing the amount of aluminum phosphate in the vaccine.

In general, no further difficulty was encountered until poliomyelitis vaccine was combined with the triple antigen product with the exception of one special product containing alum. A summary of the composite results of 498 lots of adsorbed vaccines tested over a five-year period are given in Slide 1.

May I have that, please?

It is shown that weight gains and deaths for the single, double or triple antigen products containing

either alum or $AlPO_4$ were essentially the same with the exception of the one special product. That is the one where we had seven lots. With the latter, the weight gains were lowest and death rates highest. The highest weight gains and lowest death rates were obtained with products containing $Al(OH)_3$.

With the quadruple antigen products, the weight gains were slightly lower and death rates higher than with the routine products without poliomyelitis vaccine. Several lots were rejected or withdrawn from consideration for release because of toxicity.

Slide 2, please.

The next slide gives a composite picture of the results of toxicity tests for the plain and the adsorbed products, exclusive of DTP-P, covering the 9.5 years. The data were divided into four periods for analysis.

Throughout the first three periods, the same test doses were used, that is, 1½, 5 and 1. In the last period, covering two years, the latest revised test dose of one-half the single human dose was employed. This change represented a decrease in dosage for the adsorbed vaccine and an increase for the plain vaccine.

It is of interest to note that there was a significant change in the weight gain of the control mice. At this time the Division, which was three years ago, moved

into a new building which provided improved animal holding facilities. Recently, Dr. Cohen reported that diet influenced the weight gain of mice given pertussis vaccine.

The first period of 1 and 1/2 years covered the time, mentioned earlier, when difficulty was being experienced with $AlPO_4$ adsorbed vaccines. It may be seen that the lowest weight gains with these vaccines were obtained during this period. During the next six years the gains were greater and quite uniform. With the decrease in the test dose in 1961 there was an increase in gain.

In spite of the increase in dosage for the plain vaccines, the average weight gain during the past two years showed little change.

Finally, it is of special interest to note in this chart that during the seven and a half years before the decrease in dosage of the adsorbed vaccines, the average weight gains were exceeding the 3 gram weight limit except for the $AlPO_4$-containing product during the first period and for one special product containing alum.

That is, the average weight gain obtained with the products in general were meeting the minimum required gain of 3 grams effective since August 1954.

In addition to the routine tests, a study has been made of the effect of greater doses of vaccine on

seven-day weight gains of mice. No less than three lots of each manufacturer's products, except for the DTP-P, were tested using three doses, and not less than 30 mice per dose. The combined results obtained with the products containing the three adjuvants and the plain products, respectively, are shown in the next slide. (Slide 3)

May I point out these points are in error. They should be preceded by a decimal point.

One plain vaccine was omitted since no dose response was obtained, which was the straight line. The weight gains being similar at all doses. The slopes of all these four lines are similar and not significantly different.

Slide 4, please.

In Slide 4, the regression curves for the individual products are shown. Note that the curves of some plain products are significantly different from those of others. The same is true for the $AlPO_4$ adsorbed vaccines.

Also note that each product would have met the required weight gain of 3 grams at the former dose of one-fifth the total human immunizing dose which was three-tenths ml.

Consistency studies. The results of the toxicity test of each manufacturer are being followed for consistency. The seven-day weight gains with consecutive

lots of DTP-P adsorbed vaccines of two manufacturers are given in the next slide. (Slide 5) For each manufacturer there are three horizontal lines. The solid line represents the median weight gain for the five-year period 1955 through 1960. The long dashed line represents the median weight gain for the one-year period 1960-61. And the short dashed line represents the weight gain at the 0.25 ml. dose level on the regression curve for that particular product.

Each manufacturer had his own regression curve computed, and this was in this case what this particular manufacturer got.

New median weight gains are being constructed for each product.

The dots represent the seven-day gains which have been obtained in the tests of consecutive lots since computation of the regression curves. Each time we tested a new lot, the weight gains were plotted.

With the product given on the lower half of the chart, each of the median lines is above 3 grams.

This product would have had no difficulty in meeting the 3 gram minimum gain under the old dosage of 0.3 ml., whereas the second product at the top would have met the 3 gram gain in 1955-60 but not in 1960-61. With a dosage change to 0.25 ml., the weight gains have been consistently not less than 3 grams.

May I have the lights, please.

With the use of a single strain of mice, it has been possible to obtain an overall picture of the toxicity of pertussis vaccines in the United States during the past 9.5 years. As a whole, results have been remarkably consistent. When difficulty was encountered, it was limited to the product of an individual manufacturer and not general.

The results obtained in our laboratory show that during this period the mice were usually gaining not less than 3 grams in seven days even before the 3-gram gain was specified. Since the change in 1961, weight gains have increased. Part is due to a decrease in the size of the test dose. A significant contributory factor is, no doubt, the new requirement that the bulk bacterial suspensions pass the test before incorporation in the final lot.

Our favorable results indicate that the test dose of 7.5 opacity units instead of 10 might be adequate for the bulk suspension. It is gratifying that not a single lot of vaccine has been rejected because of the toxicity since the revision in the freedom-from-toxicity test in 1961.

On the other hand, certain manufacturers have encountered considerable difficulty with the new requirement. From others there have been no complaints. It

appears that the difficulty has been due largely to the strain of mice employed by the manufacturer.

The influence of mice has been described in previous papers given at this meeting. To control variability encountered between laboratories, some workers have proposed a standard strain of mice. We consider that a stable reference vaccine for use to select the test strain of mouse would be better.

A number of questions arise concerning the selection of such a reference. In the United States, vaccines are prepared by different methods and they contain different mineral adjuvants and in different concentrations. Further, pertussis organisms contain a number of toxic factors. What toxins, and in what proportions should these toxins be in a reference preparation?

In closing, we would like to emphasize that the purpose of the toxicity test is to assure the vaccine is of low reactivity for children. There is insufficient properly recorded data on correlation of laboratory measured toxicity and reactivity in children. Some observations by Dr. Bell and a recently published paper by Dr. Cohen show a trend towards a direct relation between mouse toxicity and human reactivity.

There is an obvious need for means whereby consistent measurements of toxicity can be obtained between

laboratories. It is hoped that this symposium will contribute to the solution.

Thank you.

(Applause)

DR. MURRAY: Now, we have a slightly different presentation, on the effect of a chemotherapeutic agent and mouse strain upon pertussis vaccine toxicity test. Carl Newman and Robert Finger.

Carl Newman.

MR. CARL NEWMAN: As we have heard from previous papers, this freedom-from-toxicity test has been in use now for some ten years with just one revision in that time. I think we all have to agree that it has undoubtedly done a job. It has most certainly kept really toxic lots from the market.

As a matter of fact, attempts to correlate reported reactions in the field with laboratory test data have usually not met with success probably because there just aren't that many vaccines out on the market that have shown some degree of toxicity.

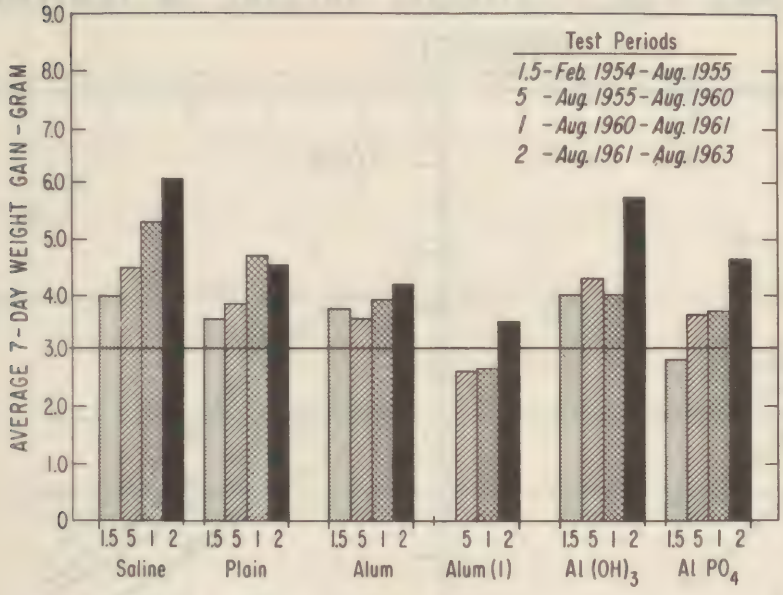
But those of us who are concerned with the laboratory evaluation of toxicity have become increasingly aware of the shortcomings of the test, and if you have been paying attention to some of the previous papers you don't need me to tell you that.

(Slide 1)

INFLUENCE OF ADSORBENTS AND POLIOMYELITIS VACCINE ON WEIGHT GAIN OF MICE, 1955-1960

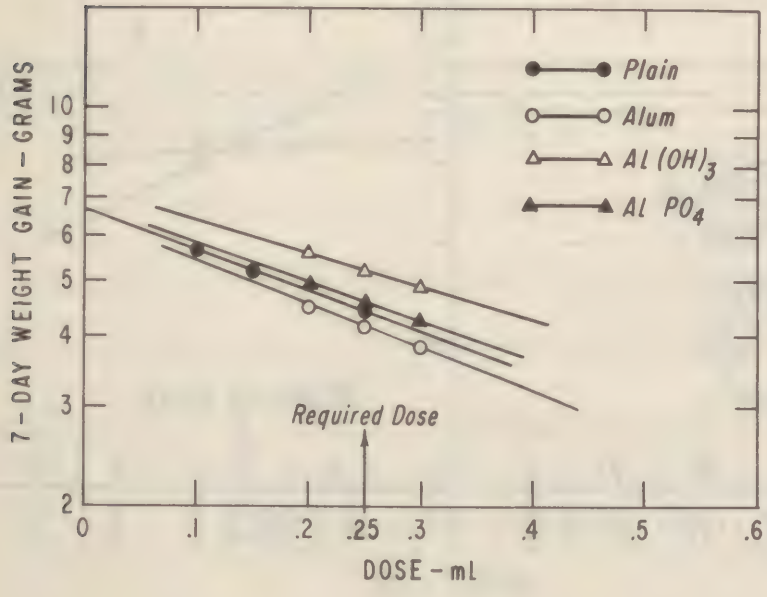
Adsorbent	No. of Lots	No. of Mice	Avg. Weight Change−g			Deaths
			24 Hr.	72 Hr.	7 Days	%
P,DP,DTP						
Alum	107	1590	−1.24	0.55	3.59	3.33
	7	340	−0.96	0.48	2.63	12.06
$Al(OH)_3$	31	420	−1.05	1.02	4.28	2.86
$AlPO_4$	276	3850	−1.16	0.74	3.60	3.32
DTP-P						
Alum and $AlPO_4$	77	1410	−1.46	0.51	3.15	4.96

(Slide 2)



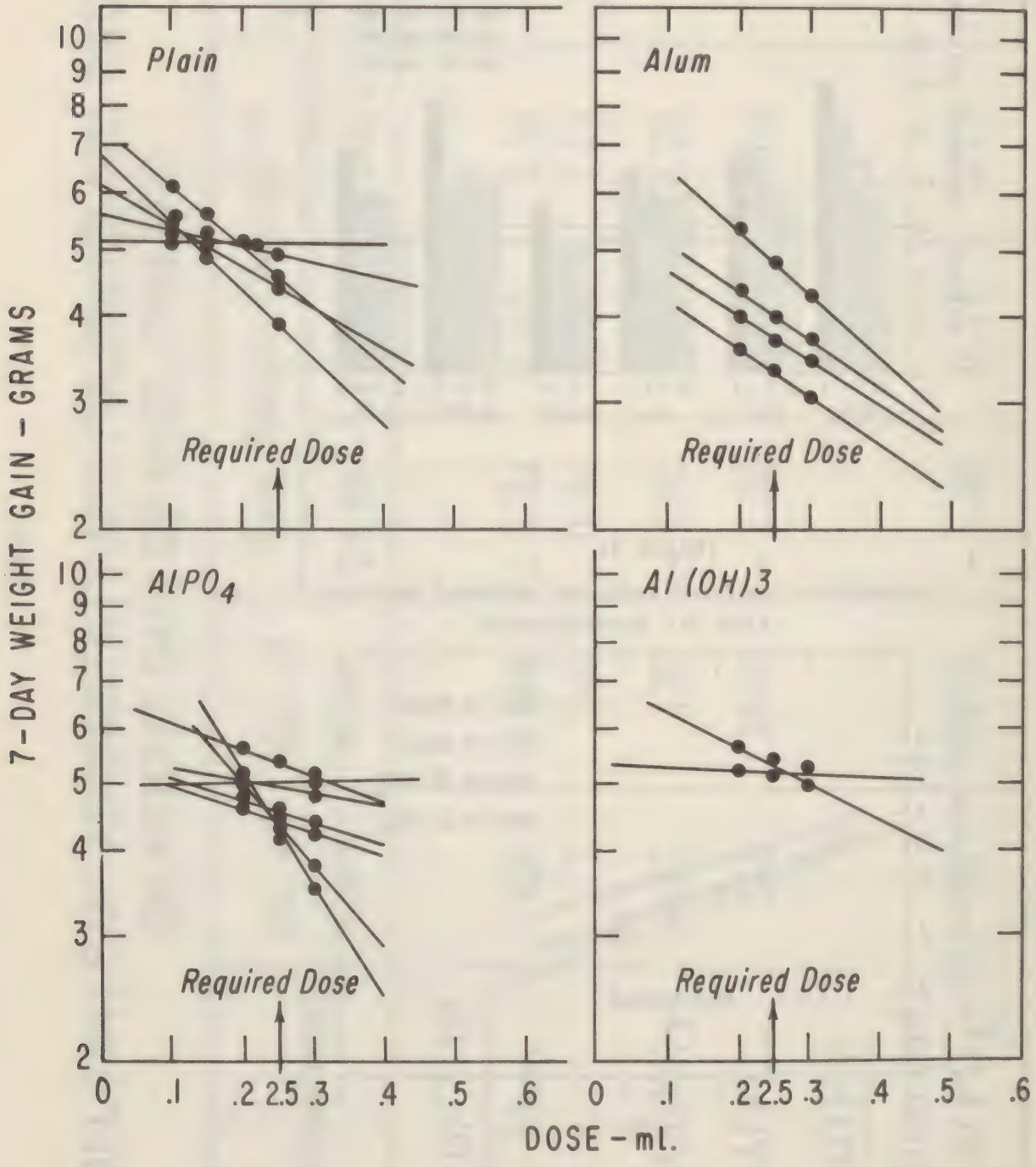
(Slide 3)

REGRESSION LINES FOR PLAIN AND ADSORBED VACCINES FROM ALL MANUFACTURERS

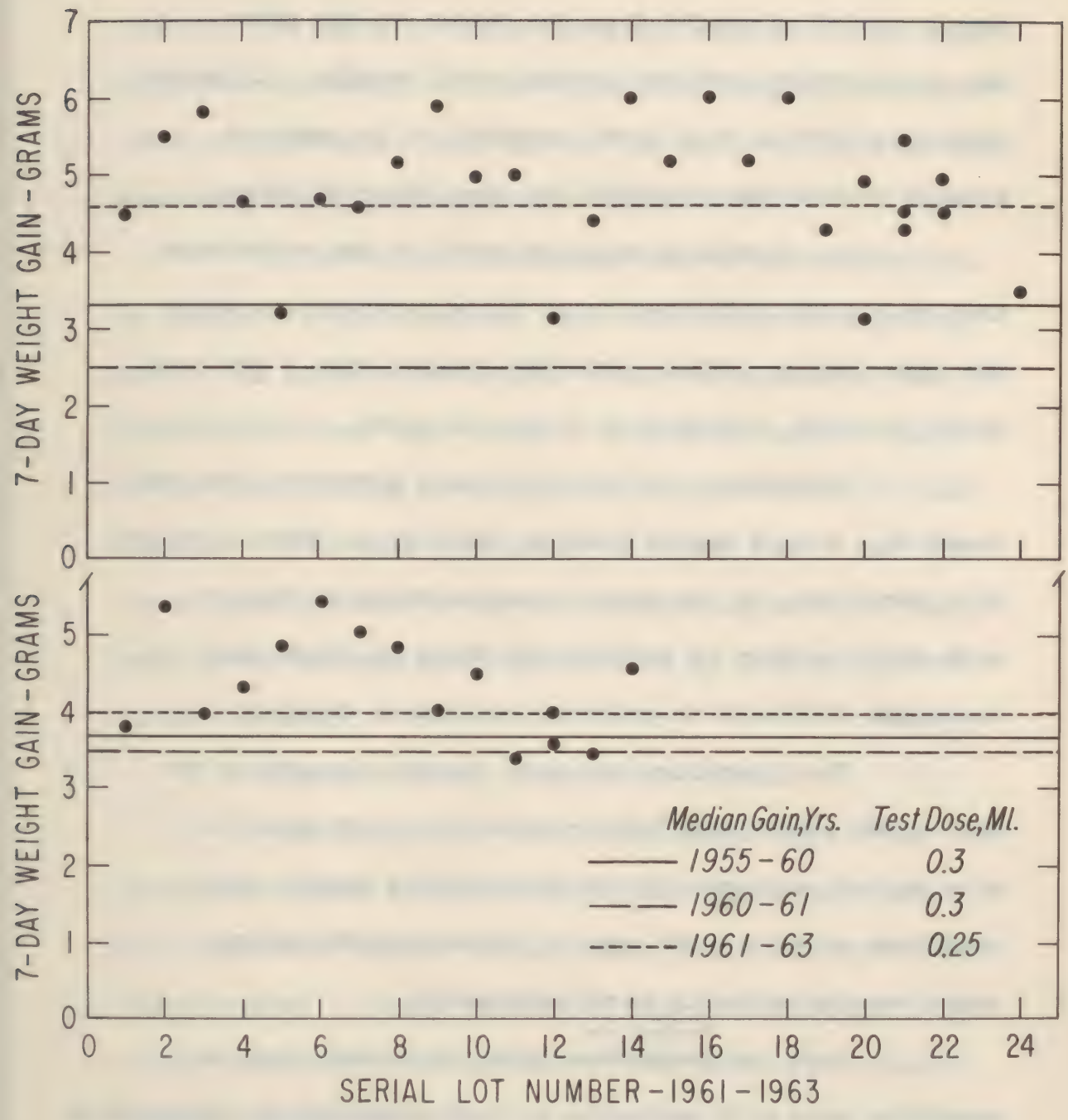


(Slide 4)

REGRESSION LINES FOR PERTUSSIS VACCINE IN INDIVIDUAL PRODUCTS



(Slide 5)


7-DAY WEIGHT GAIN - GRAMS
7-DAY WEIGHT GAIN - GRAMS
Median Gain, Yrs.
Test Dose, Ml.
1955 - 60
0.3
1960 - 61
0.3
1961 - 63
0.25
SERIAL LOT NUMBER - 1961 - 1963

Replication testing of some of these preparations over a period of months from one season to the other has occasionally produced inconsistent results. Likewise, duplicate testing on a given preparation in numerous strains of mice has produced the same thing at times.

Dr. Piersma's paper referred to the experience that we all had with that dried Lilly reference in which the same dose he listed mortality ranging from 4 per cent to 43 per cent, and this is a case in point.

Inevitably, we have all asked ourselves the same question. Are we really testing toxicity in these mice and only toxicity? Is the mouse itself a variable factor with which we have to contend and which at times makes it downright difficult to properly evaluate a vaccine?

The literature has many reports concerning the well known sensitizing characteristic of pertussis in mice to not only such things as histamine but to micro-organisms as well. And some of these reported micro-organisms are actually known pathogenic.

Arch and Parfentjev have found that they can sensitize mice with pertussis to such organisms as Pasteurella (multocida), influenza virus, and Proteus vulgaris, as well as Pseudomonas. Kind has reported the same sort of thing with the endotoxins of Shigella dysenteriae and E. coli.

And so we wondered: Are we sometimes measuring

the bacterial flora of our mice and the effect of pertussis upon it? Do our mice carry low-grade pathogens? We might call them secondary invaders which are normally innocuous, lying dormant until such time as the mouse is stressed with something like pertussis?

In an attempt to answer some of these questions we decided to pre-treat our mice with a chemotherapeutic agent. We chose Nitrofurazone rather arbitrarily. Our veterinarians had been using it with some apparent success in the conditioning of monkeys and the treatment of other animals. It is readily soluble in water, and it is easily administered by way of the drinking water to the mice in the recommended concentration of 3 grams per 16 ounces of water.

May we have the first slide, please?

The top part of this slide shows some of our early very modest attempts to see what this drug would do. These are various preparations, whole cultures, concentrates. I think these last two are actually triple antigens, final products. And this is the result from the untreated mice.

These, of course, were all conducted by the official DBS test, ten opacity units in the case of the pertussis components and one-half of the individual human dose in the case of the other.

The tests were done in our own mice in the winter time, and, as you can see, the Nitrofurazone seems to be doing something. The difference is even more marked at 14 days.

I might say that although the testhas always been officially seven-day duration, at this time we were holding our tests 14 days as I think many of the manufacturers were doing.

The bottom part of the chart shows what happened when we took a single lot in March, same mouse, and titered it for its LD_{50}, treated and untreated. And again we get the same effect. We get more survivals. It takes more pertussis to kill when the Nitrofurazone is present. And the difference again is even greater at 14 days.

In the beginning we didn't know quite how to administer this drug. We had a choice of either exposing the mice to it before the test and then stopping it or keeping the mice on the drug for the entire length of time. So we did it both ways for a while.

This just shows the one case down here where we kept it on for the entire duration of the test.

We soon found we got this effect with only three-day exposure, and we preferred this, of course, keeping it away from the test and the vaccine.

May we have the next slide? (Slide 2)

Here are some comparison tests done in three different kinds of mice, two different preparations, in August. Some of you will recognize this number as the now famous dried Lilly reference (indicating BD-10257).

Unlike the way our mice reacted in the winter time, in August now it seems that the effect of the drug is lost. There is no apparent advantage. In fact, here we even had more deaths on the treated mice with the Nitrofurazone. And pretty much the same with CF-1 mice. Hardly any difference.

The third strain of mouse, Detwiler, which is from a local dealer, however, produces again that same effect, lowering the deaths.

You will have to forgive us. We totaled these all up and we realize that this is something like adding apples and oranges where we have different strains of mice but we wanted to do this to get the overall trend. We did this as we went along.

Can we have the next slide? (Slide 3)

These are two preparations, one a pertussis component and one a final product made from that in two different kinds of mice in September. The main virtue of this slide, I think, is to illustrate the importance of saving a single mouse or two. This might not seem like

it's very important. But, as many of us know, a single mouse in this test which involves ten mice, five per cent permissible mortality, can very often make the difference between rejecting a lot or accepting a lot.

May we have the next slide? (Slide 4)

We did some LD_{50} titrations in September, three different kinds of mice, various preparations. The top one is a final product so that the LD_{50} dose is expressed in terms of ml. All the others are opacity units. Not a very great difference is shown here with MS&D mice. While the trend is the same, it increases a little bit where we caught the end point. Down in here there is hardly any difference with the Detwiler mice. A little bit more difference with the CF-1 males.

Can we have the next slide? (Slide 5)

Then we took a look at all the tests we had done, all the LD_{50} tests, in September in terms of their total survival rates. Three different kinds of mice. And again, unlike the way they reacted in the winter time, our own mouse received no special benefit from the drug, and surprisingly the Detwiler mice didn't either. But the CF-1 males show what is probably a significant difference, something like 21 mice in 180, a gain of 11 per cent.

Can we have the next slide? (Slide 6)

The statistician looked at the data and noted

that we had 46 paired comparisons, and he looked at these parameters in terms of, No. 1, how many paired comparisons favored the Nitrofurazone -- 22 out of the 46.

How many showed no difference? 19.

And how many favored no Nitrofurazone? And there were only 5.

And the results at 14 days were pretty much in the same pattern. Thus, this is the significance.

Can we have the next slide? (Slide 7)

This is the last slide. It tabulates in terms of survival rates all the tests that we have done in three kinds of mice. And we see the trend is still the same. The treated mice we do seem to cut down on these deaths, both at seven days and at 14 days. And application of the chi square test suggests that these differences probably could not happen by chance.

Now, we did some modest amount of culturing, bacterial culturing. I hesitate to say too much about it. We tried to get these mice soon after they died as possible. But where we isolated organisms we got Proteus quite frequently, some E. coli, (~~Arabector staphylis~~ Aerobacter, Staphylococcus) frequently.

From all of this one might conclude that the thing to do is to use germ-free mice. And maybe some day we will be. But until that time perhaps the next best

arrangement would be to clean up, so to speak, our own garden variety type mice with the use of a chemotherapeutic agent or some antibiotic. It's quite possible that the use of these materials might very well eliminate that occasional death which we get now and then which we don't think is related to toxicity and which very well might enable us to more fairly evaluate toxicity in our vaccines.

(Applause)

DR. MURRAY: Thank you very much.

I would suggest that because of the time in the afternoon we recess for approximately not more than 20 minutes to have some coffee.

(Whereupon, a recess was taken.)

DR. MURRAY: If we could come to order, it's getting late I'm afraid. There are no people outside the door at the moment, so I imagine we're losing in battle strength as time goes on.

I'll call on Dr. Culbertson for the next presentation.

DR. CULBERTSON: Dr. Murray and Gentlemen, in the next few minutes I want to go over a summary of some of the observations that we have made in our laboratory, having recognized this as a problem, toxicity, for many years.

When I first went to the Lilly laboratories I was called by the professor of pediatrics who was a close

TABLE 1

Pertussis Toxicity - MS&D Male Mice

Official D.B.S. Test — December - January — Doses: Component= 10 opacity units; Final D.P.T.= 0.25 ml.

Deaths/Total

	7 days		14 days	
	Untreated	Treated*	Untreated	Treated *
63970	1/10	0/10	2/10	0/10
65339-A	2/8	0/8	2/8	2/8
65339-B	2/8	0/8	3/8	0/8
65339-C	0/8	0/8	3/8	0/8
63581	1/8	0/8	3/8	0/8
63582	0/8	0/8	1/8	1/8
	6/50	0/50	14/50	3/50

* 3 grams Nitrofurazone/16 oz. water for 3 days prior to test

Toxicity LD_{50} - MS&D Male Mice

Lot 67082 Conc. — March

	Dose Opacity Units	Untreated S	Untreated D	LD_{50}	Treated ** S	Treated ** D	LD_{50}
7 days	10	10	0		9	1	
	20	5	5	20	8	2	25
	40	0	10		0	10	
14 days	10	3	7		9	1	
	20	0	10	<10	5	5	19
	40	0	10		0	10	

** 3 grams Nitrofurazone/16 oz. water for 3 days prior to test and for duration of test

TABLE 2

Pertussis Toxicity

August

Official D.B.S. Test

Dose = 10 opacity units

Deaths/Total

Mice		7 days Untreated		7 days Treated *		14 days Untreated	14 days Treated *
MS&D Males	BD 10257	1/20	Pass	1/20	(Fail)	1/20	3/20
	89-29-3	0/20	Pass	0/20	Pass	0/20	0/20
	Subtotals	1/40		1/40		1/40	3/40
CF1 Males	BD 10257	2/20	Fail	1/20	(Fail)	2/20	1/20
	89-29-3	0/20	(Fail)	0/20	(Fail)	1/20	0/20
	Subtotals	2/40		1/40		3/40	1/40
Detwiler Males	BD 10257	3/20	Fail	0/20	(Fail)	5/20	3/20
	89-29-3	3/20	Fail	2/20	Fail	4/20	2/20
	Subtotals	6/40		2/40		9/40	5/40
TOTALS		9/120		4/120		13/120	9/120

* 3 grams Nitrofurazone/16 oz. water for 3 days prior to test

TABLE 3

Pertussis Toxicity

September

Doses: D.P.T. = 0.25 ml.
Component= 10 opacity unit

Official D.B.S. Test

Deaths/Total

Mice		7 days Untreated		7 days Treated *		14 days Untreated	14 days Treated *
MS&D Males	93 - DPT	2/20	Fail	1/20	Pass	3/20	1/20
	93 Comp.	1/20	Pass	0/20	Pass	1/20	0/20
	Subtotals	3/40		1/40		4/40	1/40
Detwiler Males	93 - DPT	5/20	Fail	0/20	(Fail)	9/20	2/20
	93 Comp.	1/20	Pass	0/20	Pass	3/20	1/20
	Subtotals	6/40		0/40		12/40	3/40
	TOTALS	9/80		1/80		16/80	4/80

* 3 grams Nitrofurazone/16 oz. water for 3 days prior to test

TABLE 4

Pertussis - LD_{50}s

September

20 mice per dose level

Mice		7 days Untreated	7 days Treated *	14 days Untreated	14 days Treated *
MS&D Males	DPT	0.69 ml.	0.87 ml.	0.52 ml.	0.64 ml.
	91063 Pool	> 40 O.U.	> 40 O.U.	> 40 O.U.	> 40 O.U.
Detwiler Males	BD 10257	14	19	12	13
	91063 Pool	56	56	45	51
	91763 Pool	39	37	25	24
CF1 Males	BD 10257	17.5	25	11	19.5
	91063 Pool	> 40	> 40	> 40	> 40
	91763 Pool	39	> 40	28	36

* 3 grams Nitrofurazone/16 oz. water for 3 days prior to test

TABLE 5

Pertussis Toxicity - LD_{50} Titrations Performed in September

Total Survival Rates

7 days

Mice	Untreated	Treated *
MS&D Males	98/120 = 82%	100/120 = 83%
Detwiler Males	114/180 = 63%	116/180 = 64%
CF1 Males	136/180 = 76%	157/180 = 87%

14 days

MS&D Males	88/120 = 73%	91/120 = 76%
Detwiler Males	90/180 = 50%	96/180 = 53%
CF1 Males	103/180 = 57%	132/180 = 73%.

* 3 grams Nitrofurazone/16 oz. water for 3 days prior to test

(Slide 6)

TABLE 6

Distribution of Differential Pertussis Toxicity in Mice Treated with Nitrofurazone or No Nitrofurazone

Parameter	7 days Toxicity Test	14 days Toxicity Test
Number of Paired Comparisons	46	
No. Favoring Nitrofurazone	22	25
No. Favoring No Nitrofurazone	5	9
No. Showing No Difference	19	12
Significance of Difference (Sign Test)	$P<0.01$	$P=0.01$

TABLE 7

Pertussis Toxicity - Survival Rates - All Tests

7 days

Mice	Untreated	Treated *	% Increase
MS&D Males	218/250 = 87%	228/250 = 91%	4%
Detwiler Males	182/260 = 70%	194/260 = 75%	5%
CF1 Males	174/220 = 79%	196/220 = 89%	10%
	574/730 = 78%	618/730 = 85%	7%

Chi square = 8.45

Significance, P = <0.01

14 days

MS&D Males	199/250 = 79%	214/250 = 86%	7%
Detwiler Males	147/260 = 57%	168/260 = 65%	8%
CF1 Males	140/220 = 64%	171/220 = 78%	14%
	486/730 = 67%	553/730 = 76%	9%

Chi Square = 14.5

Significance, P = <0.001

* 3 grams Nitrofurazone/16 oz. water for 3 days prior to test

friend of mine at the medical school, and he said, "What about your vaccine, pertussis vaccine?"

And I said, "Well, what about it?"

And he said, "Well, I had two or three very severe reactions in children."

And I said, "Well, we test this in the standard test with mice and it ought to be all right."

And he said, "Well, you must have tested this on a damn pack rat." He said, "The way it hits these kids is something awful."

I got interested as to why Dr. Dettwiler's vaccine was bad, so I thought I'd better look at mine, at the mice, to see what happened. And my general conclusion over the years is that I have to agree with some of the previous speakers that in our laboratory we think we have evidence that the mice have a good deal to say about what we get regardless of the preparation which we use.

Now, with the advent of the germfree animal we have the opportunity of studying this toxic reaction in the absence of the bacterial flora of the colon.

As to my interest and background in this, I sort of slid into it obliquely in a way by having studied considerably the nonspecific resistance factors in the various bacilli as far as mice are concerned in combination with some of our other people in the laboratory and along

with it Xrayed mice. And, of course, we have many toxic cancer drugs which we have to deal with in this regard. And when we have trouble, as we have in some of the tests, of getting mice to live and possibly gain weight, and I haven't got too much information on that, it seems to me that we had a good deal of things in common between the mice which got this toxic pertussis preparation, and I think it is defined as a toxic preparation no matter whether it's good, bad or whatever it is in the mouse test. We have serious clinical reactions from it. And I think we ought to take a very good look at it.

Now, when the germfree mice became available in our laboratory we didn't quite know whether we should do germfree mouse tests or not for a long time, and this thing here gave us the notion that we should to find out what, if anything, we could do about this.

And if I can make this runthrough of a bunch of slides now for the rest of my time I'll show you what I think we have found.

Now, I must say that first when you go in for this business of studying mice you can't take liver, kidney, and the spleen and lungs and heart and thymus as we started out. You've got to do this (indicating). This is the head, the decalcified head and skeleton. And you have particularly got to do this intestinal part. If

you don't do this you miss half of the whole business, because the intestinal mucosa is next to the bone marrow in sensitivity to toxic agents, and the smallest dose of pertussis you give, as I will show you, causes a ~~stopping~~ sloughing of the mucous membrane of the small intestine, and if the mouse is sensitive by having already infection due to any of the things Dr. Newman mentioned, I think you get a Schwartzman reaction in the large bowel, and following this within seven days you get ulcers, and following that you will get invasion of the mouse, and this is responsible for those occasional deaths in my opinion.

I will try to develop that as I go along.

That was a ~~normal~~ normal/mouse, a classical mouse, I showed you.

Here's a germfree mouse. You see the colon is very large in these animals, and it constitutes a little trouble when you do intraperitoneal injection. You're apt to hit it. But I don't believe it has contributed too much to our difficulties here.

So on every mouse -- and I don't know how many hundreds of them we have run through -- we have tried to come to some conclusion about what was happening, and we're not sure yet.

I must hasten to say that as you will see-- Next slide, please. Now, you first have to rule out

spontaneous disease in mice, and from many mouse sources you will get mice from time to time showing up these things. Here's one that's got a granuloma in the jaw, an ulceration of the intestines, ulceration of the tongue and metastases of (cancer) to the lung.

This continually crops up in every source of mice that you can have. I'm convinced that nobody is completely free from it.

Next slide, please.

Now, in the upper respiratory tract-- That is the normal here, but you see mice with marked (coryza). Here's a nasal passage in a mouse. Here's the normal. This mouse has got a bad cold, a really bad one.

Here are the external ears.

Now, unless you do this in the mouse you do not know in a conventional mouse when he dies what he died of. I don't think that we can any more inject a mouse with X substance and then write down on the paper the mouse died of what we injected it with. I don't think we can ever tell that unless we look.

And these (coryza) mice particularly when you give pertussis toxic material, you get otitis media in a fairly high per cent. You always get fluid even in germfree mice in toxic dose. But here you have got a typical middle ear infection.

Next slide.

Now, here is pseudotuberculosis coming up from one of the best animal outfits in the country. Here's the lesion in the legs. There are the bacteria, bacteria of pseudotuberculosis.

Those are mouse diseases you have to deal with. Those are pathogens.

Next slide.

Now, while we have been working on this, Dr. Lagrande and other people in the histologic field have studied intestinal mucosa to a great extent. I hope most of you know about this, that the cells are generated down here in the small intestine in a mouse, in about four days they're up here (indicating), and they go off the end like this.

When you put in a toxic agent you speed up this process. And this is a mouse that has had some toxic -- I think this was a cancer drug probably -- and it shows these cells coming off the end here in visible numbers. When you get pertussis vaccine this comes off not in a few cells but it comes off in sheets.

Next slide.

When you give live pertussis I just want to show a few representative pictures of some of the changes. This is normal liver. Over there you see

vacuolization. This occurs within 16 hours after you give it.

Here's normal heart. This is heart with eosinophilic degeneration. This is a common finding in the mouse.

Next slide.

The spleen of a normal animal shows beautiful germinal centers. This is kind of a hazy picture, but this is the way it looks. These beautiful lymphocytes are stained lightly here, have undergone what we call (lympholysis). And out in the periphery here we see some hemorrhages.

The thymus has a (cortex) here that's dense. In the normal mouse after they get pertussis or any endotoxin preparation, you see the lymphocytes are all gone. This happens in two or three days as a rule.

Next slide.

The mice that die in a single day don't show much change in thymus.

Now, that's the general picture. Here's just another one showing the vacuolization. Nobody knows what those vacuoles are. They don't know whether it's fat or not.

Here's heart muscle.

Here's a mouse dying within less than 24 hours.

Here's the way mucous membrane starts to come off, and in a few hours the whole lumen is filled just the way it is here.

Next slide.

In the germfree mouse the normal picture is as follows. Here are the villi with very little inflammatory material. The liver pale staining and normal. The germinal centers. The wall of the colon here is thin and no inflammation in it.

The next slide will show us what happens in a conventional mouse in the large intestine after some of these things are injected and what happens in the germfree. This is the same section I had before. About two days after we give the toxic pertussis material, this is the germfree intestine. It thickens up. Some of the cells slough off. But notice there are no bacteria, and nothing happens.

This is the conventional mouse. The lumen is full of bacteria. Could be Pseudomonas. And here in six or seven days you get ulcers from which the animal dies of septicemia.

Next slide.

Now, a germfree mouse, those toxic changes in the liver. This is a rather quick death. I don't know the exact number of days. The first two or three days. You

see the eosinophilic changes in the dead liver cells. The kidneys show vacuolization of the cells here. This is a mass of cells sloughing off in the small intestine. This is the large intestine that shows better than the other one the sloughing of the cells here but no bacteria here.

These mice survive for three or four days and they don't die.

I think if there is any point I want to make in this discussion it is that I think there is a vast difference between what happens in the first three days of this test and what happens in the last three days up to seven, because basically the delayed deaths in my experience are practically all ulcerations of the colon and bacterial invasion, and this leads me to suggest the possibility as we go along -- you think about it -- that maybe we ought to give more material to these mice and kill them quicker and cut our tests off quicker so we don't get into these situations where we get ulcers in the colon depending on what kind of bacteria are in there. And I think there is where we are testing the mice.

I don't think strains are necessarily as important as what kind of flora they have. Dr. Dubos' work I think supports that view.

Next slide, please. (Table 1)

Now, just some quick tests through what germfrees

do and what conventional mice do. Now, if we had done this experiment and could have shown you-- It would be wonderful. We'd say use germfree mice, none of them die, you see, 25 units, and here we have the first row dead, and then the second, and this is wonderful. I thought: Let's see how far they will go.

Next slide. (Table 2)

If you do this enough you don't get the same results. The germfree mice are fairly resistant. I won't go into the fact some of these are not quite comparable weights. When you're doing germfree mice you won't have actually what you want. These mice were on outside diets. These mice were on sterile diets. Here again you see quite a difference.

Next slide. (Table 3)

Now, here's one where we ran it up to 50 units, and you see the quick deaths up here. And this is what I mentioned a minute ago, the possibility of doing tests for absolute toxicity. Don't delay and depend on killing the mouse on the basis of what he's got in his colon but depend upon the actual toxicity of the amount of this material it takes to throw him into shock. It might be a more repeatable thing.

Here again we start up at 50 and down here at 30. So this looks like it's more deaths up here in proportion

but it's not tremendously different.

All right. Next slide.

I'm having to hurry along on this because I have too much material. I want to show you the next slide I believe, what happens when you have got mice that have got already an infection. And some of our mice have had this.

Here is liver before anything was injected. And you could find this in the control as well as the injected mice. The spleen. And hemorrhages. And if you look carefully you find plasma cell masses and so on.

And over here in the intestine, down in the glands, these are masses of bacteria, and I think this mouse is already infected before we ever injected him.

The next slide shows I hope what happens. Well, this is the saline control, just to show you they are somewhat alike.

(Table 4)

The next slide/I think shows the table on this. Well, this is the bacterial stage. Here's polymorphs here. These are bacterial masses in the lumen. And you can find some of these in the mucosa.

We won't dwell on that too much because we don't have any cultures here and I don't know what these organisms are.

Next slide.

Now, here is-- I'll come back to this subject in just a minute. Those other slides are just a little out of place I'm afraid. But here I'll show you that no animal is germfree. In this we found salivary gland virus.

Next slide. Typical (cytomagalic inclusions). Here's the liver with the same picture.

Next slide. It didn't seem to affect this test. (Table 6)

Here's the thing/I want to dwell on. Here is a group of tests, two of them I think coming up, that showed that resistance business that I mentioned a minute ago, with the indwelling infection. And here you see the tremendous resistance of these animals. Yet there is one that died, and he died of an ulcer of the colon on the seventh day.

Germfree probably gave us a better picture of the toxicity of this material than did these mice because I think you could probably give them 30 units or 40 and maybe even pass it, and yet with the germfree it kills the mice.

Next slide, please.

That's the way No. 7 mouse looked. Here's the normal small intestine, but over here probably a Schwartzman reaction started this because when you inject any amount -- this is not dose-related -- any amount of this in the toxic material, I can show you other slides I don't have here but within a few hours after you inject some of these you will

get hemorrhage like you have here with the beginning of an ulcer.

Next slide.

Here's another highly resistant one with the same sort of thing.

Next slide, please. (Table 5)

Now, this group of mice had what I think was choriomeningitis. These are two germfree batches of mice that had virus infections in them. They didn't seem to bother this too much but they are there.

Next slide. (Table 7)

Here's the best test we have. This was subcutaneously given. Large amounts of material. Comparable strains of mice. Both the same. Both the same diet. Notice the death rates here. Notice the freedom from deaths there.

Next slide, please.

Here are some of the ways the outside mice looked. Necrosis of the liver. Here's the masses in the small intestine. And here's the bacteria in the gut.

Next slide.

Notice practically all those were late deaths also.

Now, I have always been curious to see how toxic this material was for mice compared to otherproducts. There is a typhoid one, (Table 8) the only one we have run. It looks

TABLE NO. 1

PERTUSSIS VACCINE USED

Soluble Antigen No. 813419A

TEST DATE

12-4-62

Type of Mice	Opacity Units per Mouse I.P.	Day of Death of Mouse	LD_{50} O.U.	Mouse Strain	Weight Gains and Losses		
					Average Gm. Wt./Mouse Start	Average Gm. Wt./Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Germfree*	25	S,S,S,S,S,S,S	>25	ND 3	15.4	17.7	21.0
	20	S,S,S,S,S,S,S,S			15.9	17.1	22.5
	15	S,S,S,S,S,S,S,S			13.5	15.7	19.5
Regular**	25	7,8,9,10,10,10,S,S	22	McAllister NIH	14.1	13.5	11.9 (7)
	20	S,S,S,S,S,S,S,S			14.3	15.0	14.9
	15	6,6,9,S,S,S,S,S			14.1	14.5	14.5 (6)

*Special Germfree Diet Used.
**Purina Mouse Checkers Diet.

TABLE NO. 2 | PERTUSSIS VACCINE USED | TEST DATE

Whole Cell BD 10257 | 1-4-63

Type of Mice	Opacity Units per Mouse I.P.	Day of Death of Mouse	LD_{50} O.U.	Mouse Strain	Weight Gains and Losses		
					Average Gm. Wt./ Mouse Start	Average Gm. Wt./ Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Germfree*	25	1,2,2,S,S,S,S	>25	ND 2	13.9	14.5 (4)	19 (4)
	20	S,S,S,S,S,S,S			14.0	14.4	17.7
	15	S,S,S,S,S,S,S			14.0	15.6	17.0
	10	S,S,S,S,S,S,S			12.6	14.4	18.0
Regular**	25	1,3,6,6,7,7,8,S	15.7	McAllister NIH	14.3	13.3 (7)	16.0 (2)
	20	2,6,6,7,8,S,S,S			14.4	14.2	14.5 (4)
	15	8,8,8,9,S,S,S			14.0	14.2	13.0
	10	8,10,S,S,S,S,S			14.4	15.4	15.5

*Special Germfree Diet Used.
**Purina Mouse Checkers Diet.

TABLE NO. 3 | PERTUSSIS VACCINE USED: Whole Cell BD 10257 | TEST DATE: 2-14-63

Type of Mice	Opacity Units per Mouse I.P.	Day of Death of Mouse	LD_{50} O.U.	Mouse Strain	Weight Gains and Losses: Average Gm. Wt./Mouse Start	Average Gm. Wt./Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Germfree*	50	1,1,1,1,1,1,1,1	27	ND3	15.4	-	-
	40	1,1,1,1,2,2,S,S			15.4	20.5 (6)	28.0 (6)
	30	1,1,1,2,3,S,S,S			16.4	18.7 (3)	22.4 (3)
	20	2,4,5,S,S,S,S,S			15.7	15.1 (7)	18.4 (5)
	0 (Saline-Inj.)	S,S,S,S,S,S,S,S			17.1	21.0	24.6
Regular**	30	1,1,4,5,5,6,7,7	24.4	McAllister NIH	15.9	13.3 (6)	-
	20	S,S,S,S,S,S,S,S			15.4	15.1	15.4
	15	7,S,S,S,S,S,S,S			15.6	14.6	14.6 (7)
	10	S,S,S,S,S,S,S,S			15.3	15.5	17.1
	0 (Saline-Inj.)	S,S,S,S,S,S,S,S			15.1	16.1	17.8

*Special Germfree Diet Used.
**Purina Mouse Checkers Diet.

TABLE NO. 4

PERTUSSIS VACCINE USED

Whole Cell BD 10257

TEST DATE

5-13-63

Type of Mice	Opacity Units per Mouse I.P.	Day of Death of Mouse	LD_{50} O.U.	Mouse Strain	Weight Gains and Losses		
					Average Gm. Wt./Mouse Start	Average Gm. Wt./Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Germfree*	30	1,1,2,2,3,S,S	23	ND2***	14.0	11.0 (2)	-
	25	1,1,2,2,3,S,S			13.9	13.0 (2)	-
	20	4,S,S,S,S,S,S			14.0	12.8 (7)	16.3 (6)
	15	4,4,S,S,S,S,S			14.3	14.0 (7)	16.4 (5)
	0				10.4	13.8	16.7
Regular**	30	2,4,5,5,7,7,7	21	McAllister NIH	16.7	14.5 (6)	-
	25	2,5,7,7,S,S,S			17.4	15.3 (6)	-
	20	1,2,7,7,S,S,S			18.4	15.2 (5)	14 (3)
	15	7,S,S,S,S,S,S			16.3	15.3	14.2 (6)
	0	S,S,S,S,S,S,S			16.8	16.4	17.4

*Special Germfree Diet Used.
**Purina Mouse Checkers Diet.
***Salivary Gland Virus Observed in These Germfree Mice.

TABLE NO. 5 | PERTUSSIS VACCINE USED | TEST DATE

Soluble Antigen No. 815775A | 6-4-63

Type of Mice	Opacity Units per Mouse S.C.	Day of Death of Mouse	LD_{50} O.U.	Mouse Strain	Weight Gains and Losses		
					Average Gm. Wt./Mouse Start	Average Gm. Wt./Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Germfree*	30	S,S,S,S,S,S,S	>30	ND2***	19.4	21	20.4
	25	S,S,S,S,S,S,S		...	20	22	23
	20	S,S,S,S,S,S,S			19.5	21.2	20.4
	15	S,S,S,S,S,S,S			21.3	23.3	24
	0 (Saline-Inj.)	S,S,S,S,S,S,S			19.8	20.6	19
Regular**	30	5,5,7,10,S,S,S	29	McAllister NIH	17.4	18.1	15 (4)
..	25	S,S,S,S,S,S,S			18.3	18.6	18.6
	20	7,S,S,S,S,S,S			17.7	17	19.3 (6)
	15	S,S,S,S,S,S,S			18.1	18	18
	0 (Saline-Inj.)	S,S,S,S,S,S,S			17.3	18	19.4

*Special Germfree Diet Used.
**Purina Mouse Checkers Diet.
***Lymphocytic Choriomeningitis Virus Observed in These Germfree Mice.

TABLE NO. 6

PERTUSSIS VACCINE USED

Soluble Antigen No. 821093

TEST DATE

7-22-63

Type of Mice	Opacity Units per Mouse I.P.	Day of Death of Mouse	LD_{50} O.U.	Mouse Strain	Weight Gains and Losses		
					Average Gm. Wt./Mouse Start	Average Gm. Wt./Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Germfree*	30	1,1,1,1,1,2	18	ND 3	13.0	-	-
	25	2,2,3,4,4,S			13	11.3 (3)	-
	20	2,3,4,5,S			13.8	9.2 (4)	-
	15	4,S,S,S,S			14	13	20.5 (4)
	0 (Saline-Inj.)	S,S,S,S,S			18	21.4	25.2
Regular**	25	S,S,S,S,S,S	>25	McAllister NIH	15.3	14.7	14.8
	20	7,S,S,S,S,S			16	14.8	11.2 (5)
	15	S,S,S,S,S			16	15	14.8
	10	S,S,S,S,S			16	16.2	18.2
	0 (Saline-Inj.)	S,S,S,S,S			15.6	17	20.6

*Special Germfree Diet Used.
**Purina Mouse Checkers Diet.

TABLE NO. 7

PERTUSSIS VACCINE USED

Whole Cell BD 10257

TEST DATE

8-2-63

Type of Mice	Opacity Units per Mouse SC.	Day of Death of Mouse	LD_{50} O.U.	Mouse Strain	Weight Gains and Losses		
					Average Gm. Wt./Mouse Start	Average Gm. Wt./Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Germfree* from Creve Coeur, Mo.	50	S,S,S,S,S,S,S,S,S,S	>50	ND4	16.9	17.2	17.4
	37	S,S,S,S,S,S,S,S,S,S			16.5	17.1	16.7
	27.3	S,S,S,S,S,S,S,S,S,S			16.3	17.4	16.7
	20.3	S,S,S,S,S,S,S,S,S,S			16.8	18.2	17.2
	15	S,S,S,S,S,S,S,S,S,S			15.8	17.5	17.4
	0 (Saline-Inj.)	S,S,S,S,S,S,S,S,S,S			17	19.6	20.2
Regular* Environment from Creve Coeur, Mo.	50	2,2,4,5,5,5,5,7,7,8	19.4	ND4	16.9	17.9 (8)	-
	37	3,4,5,5,5,5,6,10,S,S			16.7	16.9 (9)	-
	27.3	3,4,4,4,5,5,6,6,S,S			17.0	17.1	-
	20.3	2,3,6,6,8,8,9,S,S,S			17.0	17.8 (9)	14.5 (6)
	15	5,7,S,S,S,S,S,S,S,S			16.6	17.7	16.2 (8)
	0 (Saline-Inj.)	S,S,S,S,S,S,S,S,S,S			16.8	18.2	18.5

*Special Germfree Diet Used.

TABLE NO. 8 TYPHOID VACCINE NO. 829803 TEST DATE - 9/6/63

Type of Mice	Million Typhoid Cells per Mouse I.P.	Day of Death of Mouse	LD_{50} Mill.	Mouse Strain	Weight Gains and Losses		
					Average Gm. Wt./Mouse Start	Average Gm. Wt./Mouse 3 Days	Average Gm. Wt./Mouse 7 Days
Regular Environment	500*	S,S,S,S,S,S,S,S,S,S	>500	McAllister NIH	14.8	17.2	18.8
	300	S,S,S,S,S,S,S,S,S,S			14.9	17.8	19.5
	187	S,S,S,S,S,S,S,S,S,S			14.9	17.0	18.7
	150	S,S,S,S,S,S,S,S,S,S			14.7	17.3	18.6
	0 (Saline-Inj.)	S,S,S,S,S,S,S,S,S,S			15.5	18.2	19.0

*$1/3^{RD}$ the total human dose.

mild. And I never regard typhoid vaccine as completely innocuous material. So evidently our present level of pertussis vaccine has a fairly good toxic effect.

Thank you.

(Applause)

DR. MURRAY: Well, this concludes the first part of the afternoon's program, and we certainly have had a number of stimulating glimpses into what the problems are.

Now we have a rather short session on the relation of laboratory measured toxicity to clinical reactions, and I would like to call on Dr. Peck to make the first presentation, a comparison of pertussis vaccine toxicity in mice and in man.

DR. PECK: Dr. Murray, I should get out my handkerchief. I don't know which one of us is going to use it. (Laughter)

The true test of any laboratory system for detecting the capacity of a substance to produce potentially harmful effects rests in a positive correlation of the results of the laboratory procedure and the actual clinical data derived from use of the material in humans.

It is only in this manner that meaningful tests for the safety of a product can be developed and applied to assure the safety of future batches of the same material.

Minimum requirements for commercial acceptability of pertussis vaccine include a test designed to show freedom from toxicity. This implies that pertussis vaccine lots which pass this test are satisfactory for clinical use.

And, conversely, failure to pass the test automatically prevents the material from being marketed on the basis that it is a toxic vaccine and thus unacceptable for human use.

Now, the development of this test and its subsequent modifications has undoubtedly resulted in improvements in the vaccine. Of course, this includes proper strain selection, refinements in the culture media, and processing of the vaccine so that present-day pertussis vaccines no longer contain dermo-necrotic or other harmful toxins, at least that we know of.

The question, however, is whether the pertussis toxicity test truly measures the reactogenic potential of today's vaccines.

For the past 13 years we have been engaged in the development of an improved pertussis antigen, and we have had occasion to examine the reliability of the toxicity test as it applies to modern vaccines.

Now, in the clinical sense, toxicity of a vaccine is measured by local constitutional or allergic reactions

which may, of course, be potentially harmful to the recipient. Local reactions consist of an inflammatory response of varying intensity up to and including the formation of draining abscesses. Constitutional reactions usually have their onset within six to 24 hours after injection and consist of malaise, fever, irritability and the more severe manifestations such as convulsions or encephalitis.

Allergic reactions should manifest themselves by the usual dermal or systemic systems of allergy, including those rare cases of demyelizing encephalomyelitis.

In our studies we have paid particular attention to the above-mentioned reactions as indices of toxicity of pertussis vaccines, since if the vaccine is producing none of these reactions it would be completely innocuous.

Our clinical studies have been primarily concerned with the effects of injection of extracted pertussis antigen or EPA in the slides, and vaccine is composed of whole pertussis organisms or whole cell vaccines, and in the slides this is WCV.

Both pertussis antigens were used in combination with diphtheria and tetanus toxoid as DPT and with alum adjuvant preparations.

For today's presentation I will refer to whole cell vaccines only to compare their toxicity in our studies to the toxicity of extracted pertussis antigen. Correlation

of mouse and human toxicity will be confined to extracted pertussis antigen.

Now, as a preface to our results, we should mention that the mouse toxicity tests were performed and reported according to the methods in practice at the time of the tests. Some lots were tested by more than one modification of the test and are reported as either passing or failing depending on the test requirements at the time.

Reactions in patients were evaluated by the following routine:

At the time of injection, the mothers were instructed to report any local reactions occurring within three to four days after the injection, and, in addition, the injection sites were examined at the next office visit for evidence of delayed reaction such as granuloma formation.

Systemic reactions were recorded by having a mother take rectal temperature three times in the 24-hour period following injection at approximately six to eight hours -- in other words, the evening of the day they were injected and then twice the next day, in the morning and in the evening.

All parents were contacted either by telephone or by return office visit to obtain information concerning the

occurrence of fever or other immediate or delayed local or systemic reactions.

For the purposes of classification, any fever over 100 degrees, rectal temperature, was considered a systemic reaction.

Now, a summary of toxicity test results and clinical reactions following injection of EPA is presented in the first slide.

Nine lots of EPA were studied in our clinical trials. The first lot, P-68603, was made by a procedure which was slightly modified from the rest of the lots but is included because of toxicity test results.

As can be seen, in only two instances were as many as 50 per cent of the toxicity tests successfully passed, this one and this one (indicating).

None of the ten tests were passed here, and, incidentally, these are ten-mouse tests. None of the toxicity tests were passed here. None of the three here. Only one out of eight here.

On the surface, this makes a rather dismal picture of the extracted antigen, until one examines the results of the clinical tests.

Here the systemic reaction rate was quite satisfactory, the highest rate being 20 per cent of the doses producing some type of reaction.

Of over 3,500 injections reported in this table, only two produced what were considered to be severe reactions. One of these was in a child with a history of five previous febrile convulsions who received a dose of extracted antigen, developed a fever to 102 degrees, and promptly convulsed a sixth time.

The second child developed a temperature of 104 degrees and had a severely swollen arm due to a local reaction.

Both recovered uneventfully.

Slide off, please.

Local reactions, of course, are difficult to evaluate due to differences in technique of injection and injection sites. For instance, we know from our studies that fewer injections produce local reaction in the buttock than they do if they are given in the triceps or deltoid area.

However, our average local reaction rate from all our investigators using different sites if they are lumped together shows the incidence is approximately ten per cent developing some erythema or induration.

These reactions usually consisted of a tenderness and mild induration lasting less than 48 hours. No purpuric or dermo-necrotic reactions were noted. One sterile abscess was reported due to subcutaneous injection of the vaccine,

and that more of the latter were not produced is probably due to proper technique of investigation by the investigators.

Now, if one compares the reactions produced by the first two lots of EPA you saw in the previous slide -- (Slide 2) can we have the next slide? -- to the reactions produced by whole cell vaccine in our studies, one notes some startling differences. Under similar conditions of reporting, the whole cell vaccines that were employed produced significantly higher reaction rates, being on the order of four to five times more common than with EPA.

The whole cell vaccine data, by the way, was derived from results using four different commercial vaccines, and, unfortunately, the potency and toxicity test histories on these lots is unknown. There are a total of about eight lots used here. Although since they were purchased on the open marketplace they were considered to be satisfactory from a potency and toxicity standpoint.

Now, another thing concerning this data is that potency and mouse toxicity are not necessarily directly proportional, as you can see by the laboratory data shown for these two lots, one having a higher mouse toxicity, if one can say that, and yet a lower potency, essentially the same febrile reaction.

Now, the final test of any product is how it

performs in broadscale field use. Although little can be gained from evaluation of isolated reaction reports, a compilation of these reports derived from many lots of vaccine gives some indication of the reactive potential of the vaccine in question.

Obviously not all severe reactions are reported to the manufacturer, but a continuous monitoring of reports does give us some idea of whether an acceptable product is being distributed. (Slide 3)

The final slide compares the data we have tabulated on two DPT products on the basis of the total number of reports we have received versus the number of doses we have distributed.

During the five-year period from January 1955 through December 1959 we received one report of reaction for every 15,600 doses of Tridipogen alum precipitated. Tridipogen AP, as you probably are familiar, is our old whole cell DT preparation with an alum adjuvant.

Now, since the beginning of distribution of Trisoligen, or our extracted pertussis antigen, our incidence of reaction reports has decreased to one per 79,300 doses, or about one-fifth the rate of the old whole cell product.

This difference in rates is quite comparable, if you will recall, to what we noted in our clinical

trials where we compared under old conditions the two vaccines.

We think this even may be a little bit high, because with any new product you have a tendency to get more correspondence. But there has been a fairly level number year by year of reports of reactions.

Now, as is evident from our data, extracted pertussis antigen is a more benign vaccine in humans than is whole cell. However, the mouse toxicity tests indicate that individual batches of vaccine are toxic when in fact they are not, as demonstrated by clinical use.

This test does not appear to reflect the relatively benign nature of extracted antigen in humans, nor does it appear to us to be capable of showing gross differences in the reactogenic capacity of different types of vaccines.

The new Federal regulations regarding experimental drugs, including vaccines, specifically state that the manufacturer must furnish to the Federal agency involved and to each investigator, and I quote, "adequate information about the preclinical investigations, including studies made on laboratory animals, on the basis of which the sponsor has concluded that it is reasonably safe to initiate clinical investigations."

In the light of these new regulations, the failure

of correlation of the mouse toxicity test with clinical safety poses an interesting dilemma to those of us concerned with the development and future clinical testing of new pertussis vaccines.

Thank you.

(Applause)

DR. MURRAY: Thank you very much, Dr. Peck.

Now we hear from Dr. Ichter on virtually the same area but not the same products.

DR. JOSEPH ICHTER: I think Dr. Peck has defined most of the terms that I will refer to, so I don't see any need to reiterate that, so I will just talk briefly about the experience we have had with whole cell pertussis antigen only.

I'll talk primarily about DPT Trinvac and DPT plus Purivax.

If I could have the first slide, please.

This is one particular lot of pertussis which gave the following results in the laboratory testing of particular lots. As you see, on the first test, when tested as an individual vaccine, it passed. It passed again. Then it was combined to make DPT and it failed. And it failed. And it failed. And it failed. And finally they retested it again. I guess they figured if they kept going they'd finally make it. They switched from the male to the female

(Slide 1)

SUMMARY OF TOXICITY AND CLINICAL REACTIONS TO EPA

Lot #	Toxicity Tests Passed	Systemic Reaction Rate
P-68603	0/10	10%
P-68640	3/8	17%
P-68688	1/5	13%
P-68689	1/8	15%
BD-10112	2/8	4%
BD-10168	1/4	12%
774611	2/4	12%
776938	0/3	13%
776939	1/2	20%

(Slide 2)

Vaccine	# Doses	Toxicity Tests Passed	Average Potency	Febrile Reaction Rate
EPA P-68603	2712	0/10	10.8 u	10 %
EPA P-68640	306	3/8	30.0 u	17 %
WCV (A)	1200	*	*	48 %
WCV (B)	371	*	*	58 %

* presumed to have satisfactory potency and toxicity

(Slide 3)

Commercial WCV and EPA
Comparison of Reaction Reports

	dose rate
WCV	1 per 15,650
EPA	1 per 79,300

-- I think that's significant -- and it finally did pass.

Then this same biological combined with polio vaccine, Purivax in this particular instance, also failed, failed, and again finally passed.

Well, on the basis of the passing test, I was asked to take this out into clinical trial in children. Well, I wasn't particularly enthusiastic about it at this point, but I said we'd try and try it in a small number to start with.

So could I have the next slide, please? (Slide 2)

I found I get the most reliable clinical toxicity data in my experience from private pediatricians working with mothers. I have done studies such as Dr. Barrett reported where you send nurses out, work in public health clinics and situations like this, and it doesn't seem to me you get the same watchfulness of the child as the mother provides for the child.

The other thing is the final use of the vaccine is going to be with the practicing physician and the mother.

Anyway, this is the data sheet that we used. We were comparing DPT and Purivax, and the DPT by itself, in this particular study. We used rectal temperature, although we did give them an opportunity to choose, but I think 99 per cent of them came back reporting rectal

temperature.

We asked for local reactions, as you can see, redness, swelling, irritability, nausea and vomiting and any other reaction.

Next slide, please. (Slide 3)

There were five clinicians who participated, and there were more data than this but we eliminated all data we couldn't pair. By that I mean if we did not have a two-month-older who was receiving first injection of the combined biological and one who was receiving first injection of DPT from the same clinician, we eliminated that pair. So this is only paired data.

Now, after the first six hours prior to inoculation I'll say all these children had to be less than 100 degrees rectally before they were put into the study. So they were all well children. And anybody less than 100 degrees was considered well and put into the study.

This is six hours post-inoculation, rectal temperature taken by the mother. As you can see, there is pretty much agreement among investigators, and there is only occasional fairly high temperature, one here, one here, one down here.

Next slide, please. (Slide 4)

This is 12 hours after the inoculation, and again there is nothing over 104, mostly 100 to 101.9. Again

all rectal temperatures.

Next slide. (Slide 5)

This is 18 hours after injection, rectal temperature taken by the mother. No 104's. A few in the 102 to 103.9 Mostly 100 to 101.9. These are essentially afebrile cases.

Next slide. (Slide 6)

This is 24 hours. Now, the mother was instructed to bring the child back to the physician at this time, and the mother had taken a temperature at home -- 24 hours had elapsed -- or temperature was taken by the nurse in the office at that time.

Next slide, please. (Slide 7)

This will sort of give you a bird's-eye view of how this thing went, and this is six hours post-inoculation, 12 hours, 18, and 24 hours post-inoculation, and this here is less than 100 degrees. This is the afebrile group essentially. And then this is the 100 to 101.9, and soforth.

You can see that the maximum number of febrile children occurred six hours post-inoculation, and then it gradually decreases.

We followed these children longer than 24 hours, and no one who was afebrile at this point became febrile after that, and this gradually came down until everybody was afebrile.

But this would indicate to me that if you do a study such as previously reported where the children come back in 24 hours only or the nurse goes out at 24 hours only, you're really going to miss the heights of the fever curve.

Next slide, please. (Slide 8)

This is just a brief summary of the other things that we looked for. We looked for redness and swelling and nodule, and there's a difference obviously in the clinician, and this fellow here is a very positive individual who sort of approaches the mother by saying, "Now, this is something your child needs. Nothing is going to happen. Don't worry about it." So he sees nothing or she sees nothing. So you get variation here.

All of these people have participated in three or four previous trials with Purivax and Tetrovax and Tetrovax modification, and so on, so they all had experience in this before.

Irritability is hard to evaluate but I do think we get a higher incidence of irritability reported from the private physiain and the mother than you would get if you were looking at it from a clinic population standpoint.

Nausea again is hard to evaluate.

I think that the temperature, which is honest-to-goodness objective measurement, is really the main criterion

that you can use to differentiate, and I think we have briefly summarized that.

That's the last slide. Lights on, please.

Briefly, I presented a small study in a group of children under the care of private pediatricians, two different biologicals, DPT by itself failing to pass the test on four out of seven cases, the DPT plus Purivax failing to pass the test on two out of three cases.

Now, I would say that with the encouragement of this small trial that we did we went out and did about a thousand kids in an additional clinical trial where 500 got DPT and 500 DPT-Purivax, and we saw no difference really than what we saw in this study. And also these particular lots were released for ordinary commercial use about nine months ago and we have estimated that mapbe 30,000 to 40,000 doses of this have been used and we have yet to have a serious reaction reported to us.

So in this particular lot, although we had difficulty getting it to pass the DBS test, clinically it was perfectly acceptable both in the clinical trials and, as far as I know, commercially.

(Applause)

DR. MURRAY: Now we come to a discussion of this session, and since we are well ahead of time here I would suggest that we take a five-minute break and then the

September 3, 1963

Pertussis Toxicity History
(Alum. Phosphate)

All MS&D Mice used		Date	Sex of Mice	Deaths/Total	Mortality	Weight Gain	Pass or Fail D.B.S. Test
Orig. test on Pert. Comp. (Dose = 10 Billion)		11/62	male	1/40	2.5%	Statis.	Pass
Orig. test on DPT (Dose = 0.25 ml. = 6 Billion)		11/62	male	0/40	0%	Statis.	Pass
3 tests during December using male MS&D mice		12/62	male	3/30	10%	Statis.	Fail
		12/62	male	2/30	6.7%	Statis.	Fail
		12/62	male	5/30	16.7%	Statis.	Fail
2 tests in Jan. (1/4, 7) using males		1/63	male	12/59	20%	Statis.	Fail
3 tests in Jan. (1/8, 14, 15) using females		1/63	female	4/80	5%	Statis.	Pass
	w/Polio						
3 tests in Dec.	75436	12/62	male	3/20	15%	Statis.	Fail
mixed aa with 3	75443	12/62	male	2/20	10%	Statis.	Fail
different polio lots	93858	12/62	male	0/20	0%	Statis.	Pass
to simulate "Ezeject"							

Pertussis Vaccine
0.180 OB12744

TRINAVAC VERSUS 'TRINAVAC' & 'PURIVAX'

PATIENT: _____ AGE: _____

Please indicate whether this is the 1st.
2nd.
3rd.
4th. (booster)

INJECTION	DURATION	SEVERITY
Date given: _____	Local _____	Redness _____
Injectable used: _____	Lot # _____	Swelling _____
Vol. _____ Lot # _____		Nodule _____
		Other
	Systemic	Irritability _____
		Nau. & Vom. ____
		Other _____

Please take & record temperature every 6 hours for 24 hours after injection.

CIRCLE METHOD			
Axillary	Oral		Rectal
6 hr.	12 hr.	18 hr.	24 hr.

GENERAL COMMENTS

Signature _____

Date ___/___/_____

Table 1. (Slide 3)

DISTRIBUTION OF RECTAL TEMPERATURES SIX HOURS AFTER INJECTION

INVESTIGATOR	NO. OF PATIENTS	TREATMENT	LESS THAN 100.0	100.0 to 101.9	102.0 to 103.9	104.0+
	25	EZEJECT	20 (80%)	5 (20%)	0	0
	25	TRINAVAC	20 (80%)	5 (20%)	0	0
	19	EZEJECT	12 (63%)	6 (32%)	0	1 (5%)
	19	TRINAVAC	15 (79%)	4 (21%)	0	0
	19	EZEJECT	13 (68%)	5 (26%)	1 (5%)	0
	19	TRINAVAC	14 (74%)	5 (26%)	0	0
	19	EZEJECT	7 (37%)	12 (63%)	0	0
	19	TRINAVAC	11 (58%)	5 (26%)	2 (11%)	1 (5%)
	13	EZEJECT	10 (77%)	2 (15%)	1 (8%)	0
	13	TRINAVAC	11 (85%)	2 (15%)	0	0

Table II (Slide 4)

DISTRIBUTION OF RECTAL TEMPERATURES TWELVE HOURS AFTER INJECTION

INVESTIGATOR	NO. OF PATIENTS	TREATMENT	LESS THAN 100.0	100.0 to 101.9	102.0 to 103.9	104.0 +
	25	EZEJECT	20 (80%)	5 (20%)	0	0
	25	TRINAVAC	20 (80%)	5 (20%)	0	0
	19	EZEJECT	7 (37%)	10 (53%)	2 (11%)	0
	19	TRINAVAC	9 (47%)	10 (53%)	0	0
	19	EZEJECT	9 (47%)	9 (47%)	1 (5%)	0
	19	TRINAVAC	6 (32%)	13 (68%)	0	0
	19	EZEJECT	4 (21%)	13 (68%)	2 (11%)	0
	19	TRINAVAC	11 (58%)	7 (37%)	1 (5%)	0
	13	EZEJECT	9 (69%)	3 (23%)	1 (8%)	0
	13	TRINAVAC	11 (85%)	2 (15%)	0	0

Table III (Slide 5)

DISTRIBUTION OF RECTAL TEMPERATURES EIGHTEEN HOURS AFTER INJECTION

INVESTIGATOR	NO. OF PATIENTS	TREATMENT	LESS THAN 100.0	100.0 to 101.9	102.0 to 103.9	104.0 +
	25	EZEJECT	19 (76 %)	3(12 %)	3(12 %)	0
	25	TRINAVAC	17 (68 %)	4(16 %)	4(16 %)	0
	19	EZEJECT	8 (42 %)	11(58 %)	0	0
	19	TRINAVAC	14 (74 %)	5(26 %)	0	0
	19	EZEJECT	11 (58%)	7(37 %)	1(5 %)	0
	19	TRINAVAC	10(53%)	8(42 %)	1(5 %)	0
	19	EZEJECT	8 (42 %)	11(58 %)	0	0
	19	TRINAVAC	11 (58%)	7(37 %)	1(5 %)	0
	13	EZEJECT	7 (54 %)	5(38 %)	1(8 %)	0
	13	TRINAVAC	9 (69 %)	4(31 %)	0	0

Table IV (Slide 6)

DISTRIBUTION OF RECTAL TEMPERATURES TWENTY-FOUR HOURS AFTER INJECTION

INVESTIGATOR	NO. OF PATIENTS	TREATMENT	LESS THAN 100.0	100.0 to 101.9	102.0 to 103.9	104.0 +
	25	EZEJECT	18 (72 %)	4 (16 %)	3(12 %)	0
	25	TRINAVAC	18 (72 %)	5 (20%)	2 (8 %)	0
	19	EZEJECT	14(74 %)	5 (26%)	0	0
	19	TRINAVAC	19(100%)	0	0	0
	19	EZEJECT	11(58%)	7(37%)	1(5%)	0
	19	TRINAVAC	9(47%)	9(47%)	1(5%)	0
	19	EZEJECT	7(37%)	12(63%)	0	0
	19	TRINAVAC	13(68%)	6(32%)	0	0
	13	EZEJECT	8(62%)	4(31%)	0	0
	13	TRINAVAC	9(69%)	4(31%)	0	0

(Slide 7)

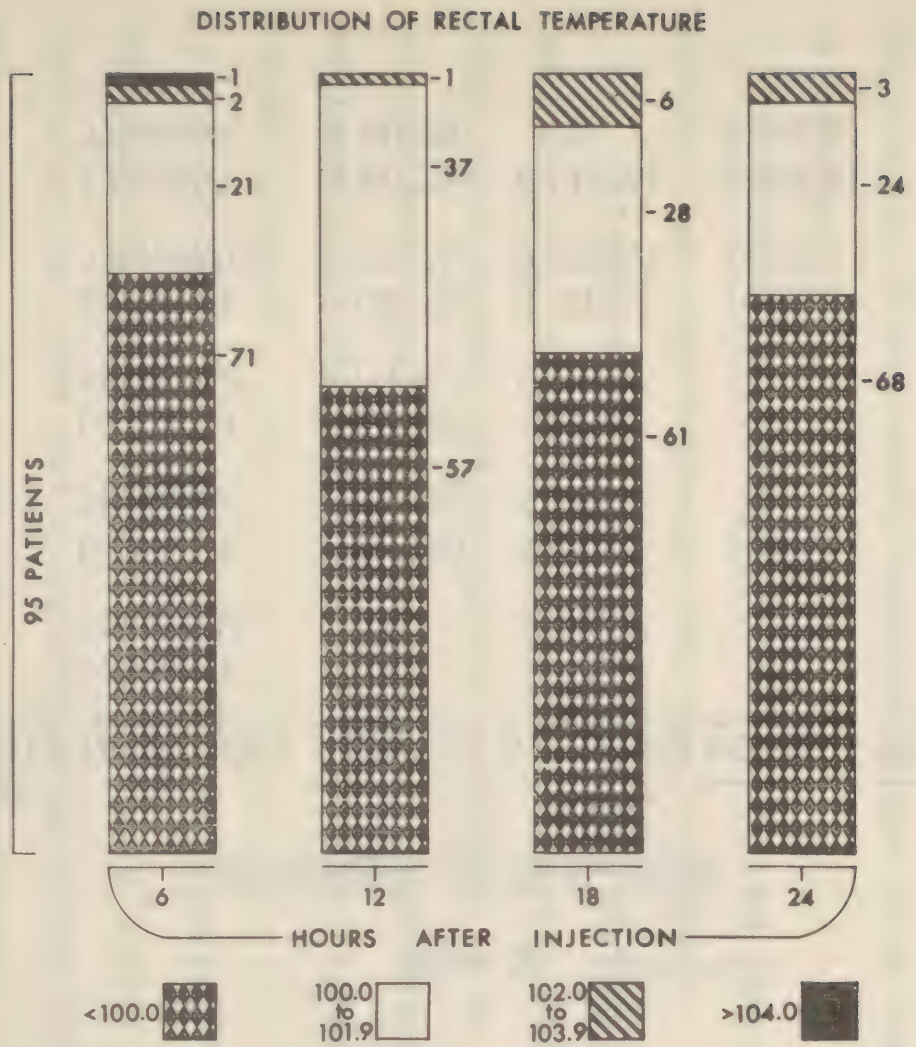

DISTRIBUTION OF RECTAL TEMPERATURE
95 PATIENTS
1
2
21
71
1
37
57
6
28
61
3
24
68
6
12
18
24
HOURS AFTER INJECTION
<100.0
100.0 to 101.9
102.0 to 103.9
>104.0

Table V (Slide 8)

INCIDENCE OF REACTIONS

INVESTIGATOR	NO. OF PATIENTS	TREATMENT	LOCAL			SYSTEMIC	
			REDNESS	SWELLING	NODULE	IRRITABILITY	NAUSEA
	25	EZEJECT	0	1(4%)	0	0	0
	25	TRINAVAC	0	1(4%)	0	0	0
	19	EZEJECT	8(42%)	3(16%)	1(5%)	9(47%)	0
	19	TRINAVAC	6(32%)	3(16%)	0	6(32%)	0
	19	EZEJECT	4(21%)	1(5%)	0	3(16%)	0
	19	TRINAVAC	3(16%)	1(5%)	0	3(16%)	1(5%)
	19	EZEJECT	6(32%)	4(21%)	1(5%)	15(79%)	0
	19	TRINAVAC	5(26%)	5(26%)	1(5%)	11(58%)	1(5%)
	13	EZEJECT	8(62%)	6(46%)	9(69%)	7(54%)	2(15%)
	13	TRINAVAC	6(46%)	0	6(46%)	6(46%)	0

discussants, those who participated in this session, come up to the head of the table and then we can proceed. I think this five minutes will give you a chance to settle down and perhaps ask some questions one of the other.

(Whereupon, a recess was taken.)

DR. MURRAY: Well, to get the discussion kicked off, I'd like to call on Dr. Brueckner to open up on behalf of industry.

DR. A. H. BRUECKNER: I don't know about that last remark. I was perfectly happy until then. (Laughter)

I only have two or three remarks, Dr. Murray, which are in the nature of additions to what we have already heard, but I thought that it might be of interest to the group to hear a little more about these mice which Henry mentioned in his paper which were not able to gain sufficient weight on saline.

We were the fortunate ones to have these mice in our premises. And we were prompted as a result of this preliminary work with just a couple of groups of ten mice to make a little further study so we obtained a hundred of these mice and divided them into groups of ten, ten groups of ten, and injected them with saline, and we found that only two of the ten groups of ten were able to reach the 3-gram requirement in the required time.

One of the other groups did make exactly 3 grams,

and one was 3.5.

So these were obviously at that time at least not good choices for testing any product.

But I think the interesting thing is that we had used mice from this same source and labeled the same strain earlier and were successful, and we have been able to find some since that seem to gain all right. So perhaps Dr. Culbertson was correct when he mentioned that maybe we are talking about strains of mice when really we should be talking about the conditions under which mice have been handled and under which they are handled in our laboratories.

There was another interesting thing that I don't know whether Henry pointed out or emphasized, and that is that even at the time when these mice were unable to gain 3 grams without product being injected, the mortality rate among these mice with injection of the Lilly toxic material was among the lowest of all the groups. I don't know whether that had any significance or not.

We have also tried to see what the effect of aluminum phosphate and certain preservatives would be on the test, and in using the phosphate relatively below the maximum limit we haven't been able to demonstrate there is any great effect on the test. However, we used only one pertussis preparation or closely related preparations.

And finally, on the subject of pathology, I think most of you are familiar with some work presented over a year ago concerning our finding that highly toxic doses of pertussis preparations appear to cause a severe dilatation of the duodenum. Since that time we have tried to answer the obvious question as to what effect intraperitoneal injection would have had on this pathology. And we have injected some mice intravenously with some toxic material and get the same picture of dilation with the same pathological picture, and this is with regular mice.

DR. MURRAY: Dr. Perkins.

DR. PERKINS: Not on behalf of the Council.

DR. MURRAY: On behalf of yourself.

DR. PERKINS: I don't wish to take up very much time because you have a different problem on your hands from that in our country, because we just don't have a toxicity test (laughter and applause). This may be being an ostrich, but we don't believe that we should put in a toxicity test unless we know more about the antigen or fraction that is causing reactions in humans.

And, of course, it's very easy to write in a test, but it's a hell of a job to shift it when once it's in the regulations.

We are doing this test, however. We have got evidence -- and it's no surprise -- different strains of

mice are giving entirely different results in this weight gain test.

We just don't have any results so far that are giving us an index of whether a vaccine would be dangerous. And when I was speaking of reactions this morning, I didn't wish to infer we were seeing more reactions than anybody else. I was talking about the neurological complications.

I don't think there is any evidence that in the United Kingdom we're having any more or any less reactions than you are over here. But, of course, we shall look pretty silly if we do get some pretty severe reactions and we haven't got legislation for toxicity test.

However, I think there are many more points you wish to discuss with your manufacturers here, and I don't wish to take up any more time now.

DR. MURRAY: Thank you.

We throw the matter open for general discussion, and any of the participants who care to make any further comments at this time before inviting a general free-for-all.

Dr. Culbertson.

DR. CULBERTSON: I don't have anything unless I go back and go into more detail, and I haven't time to do that.

DR. MURRAY: Well, for the sake of being provocative, it seems to me that there are really two problems

here. One is the problem of toxicity, and I think that some of the rather interesting findings that we have had presented to us this afternoon, particularly by Dr. Culbertson, indicate that this does exist. The problem is to measure this.

So then we come to the second problem, the problem of the test. And Dr. Perkins mentioned the story of the ostrich. But it seems to me that the way to do this is to try and measure the toxicity rather than find ways of circumventing it.

In the absence of a great deal of collateral information, some of the material presented here today may be less pertinent than it might otherwise be. I'm not saying this to be difficult. For instance, Dr. Ichter's presentation in which he showed that there was very little difference in the clinical findings despite the fact that the material failed on the mouse toxicity test.

Now, we have the age-old problem here that the toxicity test as done in another laboratory, namely, DBS, was satisfactory.

So we come back to the second of the problems, and that is the toxicity test and how we are going to conduct it and what would be a satisfactory test and how are we going to measure toxicity.

Dr. Dettwiler.

DR. H. A. DETTWILER (Eli Lilly and Company): We have had I think what amounts to a suggestion that perhaps this test, if we are going to measure toxicity, might well be changed to reduce the time and perhaps increase the dosage. And in view of the discussion that is scheduled for Wednesday on the recommendations for regulations, I'd like to recommend to those of you who are interested in that phase of it to give some thought prior to Wednesday morning to such a proposal or any other proposal, because I think we are at a point here where there are good reasons for perhaps seriously considering a change in the test technique. And we, of course, always prefer to have these things settled before regulations are written, because it is almost axiomatic once they are written it's awful hard to change them.

I believe with the thinking that I'm asking people to do we should consider how a proposed test should be checked out, because we have had evidence today that casts doubt on the validity of animal tests in relation to human tests for a variety of reasons.

DR. A. C. WARDLAW (Connaught Medical Research Laboratories, Toronto, Canada): Dr. Murray, several speakers today have said that one of the most valuable indices of reactions in infants is a rise in temperature, and I wondered if any effort had been made to measure

quantitatively the pyrogenicity of pertussis vaccines and perhaps to show if there are any differences.

DR. MURRAY: Dr. Peck.

DR. PECK: Several years ago when we were experiencing some difficulties with the toxicity test and making a decision as to whether to do this in humans, I ran a little experiment myself. I'm not a good pathologist but I think I recognize cell damage when I see it.

I used nine, as I recall, different preparations in a pyrogen test intravenously in rabbits, and these were both fluid and alum precipitated plain pertussis, DPT both fluid and alum precipitate, the soluble antigens, and anything I could get my hands on.

And without exception all of the rectal temperatures in these rabbits went to 108 and 109. About 25 per cent of them died. And on pathological examination of the organs you could find nothing except occasionally some cloudy swelling in the kidneys. We found nothing specific on this, and we could not distinguish between one vaccine and another from these things.

So that we used a lot of rabbits and our information was consistent. They all produced fever when given intravenously. That's all the information I have.

DR. WARDLAW: I was going to say a pyrogen test

is a ~~shock~~ dose/response thing, and you would have to choose the right dose range.

DR. PECK: That's true.

DR. MURRAY: Do you care to comment on that, Dr. Pittman?

DR. PITTMAN: I think Dr. Wardlaw has made the point. If you're testing at the top of the curve you don't get any difference.

DR. PECK: I realize that.

DR. JOHN J. MUNOZ (Rocky Mountain Laboratory, NIH, Hamilton, Montana): I think it's pretty well known various strains of pertussis contain different amounts of endotoxin, and I wonder if all the reactions or most of the reactions that you have described here are not due to endotoxin.

And, of course, as you well know, endotoxin is the best pyrogen that we know of. And then reactions should be considered as a result of endotoxin perhaps.

DR. DEVLIN: Posing the question to Dr. Culbertson, I wondered whether he had used pertussis endotoxin or typhoid endotoxin and as to whether the same type of histopathology occurred on endotoxin which would occur with pertussis whole cell.

DR. CULBERTSON: I can't say that my method would always be able to make fine differences, but Paul

Ensminger and some other people back home have injected animals for me to look at under various circumstances, and it doesn't make much difference whether you use ~~wholly~~ coli endotoxin or whether you use pertussis.

As a matter of fact, pertussis is a better one from a standpoint of eliciting these various changes.

And the question of the dosage, of course, has not been determined. And some of the things in the delayed deaths are not necessarily dose-related. I have a notion that a very small amount of this material, even down-- And I think you saw where the tests were of Dr. Dettwiler's sample here, some people testing that at 7.5 opacity units and got deaths. And I fancy they'd all be delayed deaths.

And I think you will find ulcers in the colon on them. Very few of those mice are going to die acutely. Whereas when we raise the dose up here to 25 we get acute deaths, and in the germfree the animals survive if they make that 48 hours. Apparently the mucosa regenerates, and that's all there is to it. These mice are perfectly healthy.

But if they have got bacteria -- and I want to stress the point it doesn't have to be a pathogen as was stated earlier -- the Xray people have found that they just can't do anything with Xraying mice if they have got any of these Pseudomonas and that group. And I think we

are dealing with the same thing.

The reason I think this, some of our people decided they'd test the mice with mucin. And I feel very stupid about this. I have watched people use mucin for a long time on the basis that this was a preventer of phagocytosis. Well, if you make a smear of the mucin preparations you will see what you're doing essentially is injecting a suspension of gram-negative bacteria which are probably nothing but endotoxin. In other words, you're just adding to the other things with an endotoxin injection.

And even that is the same picture with that mucin. And so mice get ulcers with this, if they have got Pseudomonas or Proteus, and some mice do not.

Now, I presume Dr. Pittman's mice have got a standard more or less flora, and time after time she can repeat this. But most of us who do not have such a fortunate situation are not going to be able to agree with her tests nor agree with each other, and I think this is the problem.

DR. I. MILLMAN (Merck, Sharp & Dohme): I just wonder, to be a little facetious now, if we would find better correlation with what happens clinically and what happens in mice if we would give children intraperitoneal injection.

On that score I would like to know does anybody

have any data dealing with other routes of injection other than the intravenous?

DR. CULBERTSON: We had one subcutaneous test here in mice.

DR. MILLMAN: We might get a clearer picture with a number of sites.

DR. MURRAY: Mr. Marshall.

MR. MARSHALL: Someone asked about the relative level of pyrogen. I remember we did some studies on market materials, and I think the pyrogen was diluted to get the sixteenth of a degree point which is the passing or failing point in the pyrogen test, which eliminates the objection Dr. Pittman raised of being at the top of the curve.

And this required dilution of 1 to 1,000 and 1 to 10,000 with a midpoint of 1 to 5,000 would just pass.

So that gives you an idea of the pyrogen level you're dealing with.

DR. ELDERING: To follow what Dr. Munoz said, I wonder in relation to the endotoxin-- Dr. Munoz has done so much with this factor. We have been concentrating, the people who have made vaccines, on testing the cultures we select for their mouse protective properties, but we haven't paid much attention to whether they varied in their endotoxin. We haven't chosen cultures on that basis

at all.

I wondered if Dr. Munoz could add anything to that relative to selection of cultures.

DR. MUNOZ: I'm sorry to say that I haven't done too much on endotoxins. The work that I was trying to bring to you was the work of Dr. Malmgren and Reed. And they found that some strains of pertussis contained appreciable amounts of endotoxin and others contained practically nothing.

And by this I mean they could inject, as I remember, about 15 milligrams of heat-killed pertussis into a mouse and the mouse wouldn't die. And this to me means that some strains don't have the heat-labile toxin which we call endotoxin.

As far as selecting strains according to the amount of endotoxin that they contain, I would say that we cannot ignore this fact.

DR. ELDERING: We ought to go home and look our strains over again.

DR. MUNOZ: You should.

DR. CULBERTSON: Did you say heat-labile endotoxin?

DR. MUNOZ: Heat-stable. I'm sorry.

DR. CULBERTSON: What we've got is heat-stable.

DR. DEVLIN: We did make vaccines from a strain

from Dr. Malmgren, and, unfortunately, the toxic properties were in direct relationship to the potency.

MRS. H. SKEGGS (Merck, Sharp & Dohme): I'd like to ask Dr. Culbertson if he has compared the acute LD_{50} of a whole cell vaccine with the extracted antigen.

DR. CULBERTSON: The acute LD_{50}? I don't know. Paul, have we? We have been doing some work on it lately, and I don't know how far we've gotten.

MR. ENSMINGER: We have been doing it lately on just a few mice and really there are not enough mice to make it worthwhile at the present time.

DR. CULBERTSON: It didn't make any difference, I might say, in these tests I'm talking about, in the death rate between germfree and normal. It didn't make any difference whether we used extracted or whole cell.

As a matter of fact, we used whole cell several times, and I don't think I would have been able to tell one preparation from the other in the mouse test that we gave up to 50 opacity units.

DR. MURRAY: Well, from what I have heard thus far, it seems to me that there is general agreement that there may be problems associated with toxicity, although there are baffling inconsistencies in this. But nobody has thus far said that the evidence clearly suggests that we should abandon a toxicity test.

I think at least my interpretation of what I have heard thus far is that there should be some improved methods of measuring toxicity in pertussis vaccine which are reproducible, more reproducible perhaps, and perhaps when that is done they will correlate with the clinical illness.

Dr. Pittman.

DR. PITTMAN: No one has said anything about what was the first toxicity test in 1949. This was on the bulk suspension. The test dose was 7.5 opacity units. We did not test the final preparation before 1953.

For Dr. Piersma, the famous Dettwiler preparation gave in our hands less than 3 per cent mortality. It was less than any of the reports from the manufacturers. The weight gain was not quite 3 grams. But we had the least mortality of any.

I was rather interested in both Dr. Peck's and Dr. Ichter's report. Dr. Peck had a report of incidence of reactions from different clinicians varying from 4 to 20 per cent, and Dr. Ichter, if I read the slides correctly, had it running from 15 to 63 per cent.

Well, those are very wide variations when you're trying to compare products.

It looks to me like there is more variation between the observer than we would expect between products.

And with that wide variation, what percentage of reaction should we expect in children? This is febrile reactions. 20 per cent? 10? 12? 48 per cent? What should we expect in the child?

DR. MURRAY: Dr. Ichter.

DR. ICHTER: I think the total number of reactions really has to be broken down into how many 104's and febrile convulsions do you get. I mean I as a parent and I as a pediatrician would be willing to accept 30 per cent febrile reactions between 100 and 102, but I'm not willing to accept any reaction, be it half a per cent or whatever, of 104 with a convulsion.

So just to say there was 15 per cent febrile reactions or up to 30 per cent I think is more meaningful when you look and say there was really no one over 104. I think there was one individual who went over 104 degrees at any period of time. Then you're getting into the dangerous period where the child might have a febrile convulsion.

Here we are taking a ten-pound infant, and I would guess that there are very few infants that get inoculated with this under ten pounds, and we're inoculating them on to 50, 60 or 80 pounds, giving half cc., and giving the mouse what would be like giving the infant something like 10 cc. ofthe stuff. Would it be more realistic to test your animals in a dose and a route that is used in the

clinical application?

DR. MURRAY: Dr. Culbertson had something.

DR. CULBERTSON: It seems to me that one of the things in answer to Dr. Pittman's question -- I'm not experienced in the clinical reaction rate here -- but it might depend entirely on what that investigator was dealing with, what type patient. If he had a group of patients that happened to have some of these pathogenic E. coli and been sick before they were injected, it might not be the fault of the investigator except that he didn't take temperatures before he shot them, which I think ought to be done always.

He might be dealing with an individual situation that would vary this much. It wouldn't necessarily reflect on the investigator. It might reflect more on his material.

I think this is the crux of this thing. It certainly indicates this for the mice. What the mice get depends on what they are, what's in the colon and so on.

And I can't get away from the idea that the children react depending upon what is going on in them. And I don't suppose we'll ever get a toxicity test that will show us no reactions at all.

I think we need two things. One is to have a preparation that we know is relatively uniform. And, secondly, we ought to consider how little of that stuff we

could give them to get immunity.

And there's something else that opens up another question in my mind. Are we giving them too much of this material -- the humans?

DR. MURRAY: Dr. Peck.

DR. PECK: I think I may be able to clarify my data a little bit for Dr. Pittman and the rest of you. The batch which produced 4 per cent reactions and the batch which produced 20 per cent reactions were two of five consecutive lots of vaccine that were tested by the same investigator using the same technique of injection and the same reaction reporting type of procedure.

And, incidentally, in review, the one that produced 20 per cent reaction passed one out of two toxicity tests, and the one that produced 4 per cent reaction passed two out of eight.

We still, as I say, cannot find any correlation between how this material acts in humans and how it acts in mice. And if someone wants a suggestion of whether to abandon this test, on the basis of our data we can't find a correlation, and in my own laboratory I would abandon the test.

DR. MURRAY: Dr. Wilson.

DR. WILSON: Mr. Chairman, I would like to ask some of the people who have dealt with clinical reactions

if they feel that the stress phenomenon which is evident in animals when they are injected with pertussis vaccine might not account for some of the severe reactions in children where they might have concurrent infection. How do you eliminate that in evaluating your clinical? Is there any clinical evidence of the stress phenomenon?

DR. ICHTER: The criterion which we have set up for the child to be put into the study is that the child must be examined-- That is, the child comes to the physician for a well baby checkup. The mother brings the child believing he's well, and the child is examined by the physician and determined to be as physically well as you can by history and physical examination and temperature.

If the child has an acute illness of any kind or a temperature greater than 100 degrees rectally, then he is not immunized either in our studies or by most recommendations for any particular immunization procedure.

The other thing we try to do is we always try to pair them up if possible. For instance, in the study reported we were actually trying to evaluate the Ezeject, and we took a biological which we had some background information on, so we let the physician work with two biologicals in all cases and sometimes more than two so that they would have a check. We would have a check on him

then.

DR. MURRAY: Dr. Peck, you were trying to --

DR. PECK: No, I was just going to say the same thing Dr. Ichter did. I think all of us that are in the clinical trial business give instructions to our investigators that children that are overtly ill or that we find evidence of physical illness in vaccine studies, they do not take part. And, of course, there are a lot of things that are subclinical. A lot of things go on that are subclinical. There are children incubating infectious disease, things like this, that you have no idea of.

However, in large groups of children where two things are being evaluated, the bias on one side balances the bias on the other as far as this is concerned, so we do have some unknown factors. But if the groups are large enough I think we tend to even these out.

DR. CULBERTSON: I would say a similar thing I mentioned before. Take the triple typhoid now. I'm sure if you injected as much of that into children as you do pertussis that we'd hear some trouble from it.

Now, we have no tests for this, and I don't know what you could test any more than what we have tested here. And I agree with Mr. Munoz. Probably most of these phenomena in animals are endotoxin. And yet we

have put up with this and we do have a count. That's about all we have on it, isn't it?

DR. MURRAY: Well, I have been very much struck by two pieces of information this afternoon that are new to us I think. One is the information that Dr. Culbertson has brought forward on the germfree animals which is certainly very intriguing.

And within that is the demonstration that pertussis is toxic and a rather astonishing demonstration that whatever toxicity typhoid has for mice is of a different order of magnitude.

And then the other piece of information which is a little unsettling, particularly when we talk in terms of reducing the amount of active material in an immunizing dose, is the paper that was presented by Dr. Eldering on the way that the immunity fell off in the Michigan study.

DR. ELDERING: Those were household contacts.

DR. MURRAY: That's right. But at least it takes away the comfortable feeling that the vaccine has been fully responsible for the reduction in morbidity in pertussis in recent years.

I'd like to call on Dr. Lepow at this point.

DR. MARTHA L. LEPOW (Western Reserve University, Cleveland, Ohio): I feel almost like a stranger in a strange place, because I basically work in a virus laboratory

rather than on the problem of pertussis.

The reason I'm going to speak at the moment is that a few weeks ago Drs. Kendrick and Eldering were in Cleveland and mentioned the problem of occurrence of cases of pertussis in household contacts of children at Grand Rapids which had been a highly immunized population.

Within a week of that time I was speaking to a practitioner from a rather small community in southern Ohio, and this practitioner mentioned that they had been seeing pertussis last spring in older children. This was rather unsettling to him and another doctor that worked in the same community.

Unfortunately, there's no bacteriologic confirmation of these cases. Both of the physicians were quite sure that the cases they saw were pertussis.

I went down to this community and discussed the problem with the physicians and tried to get some information from the families, and, in short, I interviewed parents of ten households where there was an illness that I think at least on clinical grounds would have to be accepted as pertussis.

The age range of children was from less than one year up through 16. As far as the case incidence was concerned, there were 19 of 28 children who had an illness with cough and paroxysms that lasted for six weeks

or longer.

In terms of the numbers of children within different age groups who had the disease, there were several unimmunized infants. There were very few cases in immunized children between the ages of 1 and 10. And of the 13 children between the ages of 10 and 16, 9 of those had a fairly full-blown illness.

Now, it's of interest in that particular group of children all of these had been immunized. There were excellent immunization records on these children in the doctors' offices. And I was quite impressed. These children had had pertussis and they had also been well immunized.

Now, the interval between the last immunization was at least six years -- between that immunization and the onset of illness this past spring. And it was unfortunate this did not come to somebody's attention at the time.

My guess would be that there were many, many more cases than these particular households.

And one additional feature. Of the 18 parents that were involved, there was at least one who had a past history of pertussis, who had an illness with paroxysms and cough that's still going on at the present time, although she said it's now getting milder. She's a school teacher in a high school class where there are three cases. It

might be reasonable to assume she was either a contact or transmitted it.

DR. MURRAY: Thank you.

Dr. Peck.

DR. PECK: I have a little scrap of data. I don't know whether it's apropos or not. But in the light of these rather alarming reports of cases of pertussis in vaccinated individuals, we had occasion to run a series of serological determinations on infants either under three months, namely, six weeks of age at the first dose, or over three months, usually four to six months at first dose, using Tridipogen, which was our old DPT product.

We found only 55 per cent of them developed an increase in titer using Dr. Culbertson's and Mr. Ensminger's modified mouse neutralization test.

In spite of this, the agglutination titer was around 85 per cent conversion from negative to positive. I don't know what this means. But it might mean that some of these children don't respond to pertussis vaccines and maybe they stay susceptible. Not all of them but some of them. And maybe these are the ones that are coming down.

I don't have the faintest idea how to go about finding this out.

DR. ELDERING: I don't see why it's so surprising that pertussis immunization wears out. Even with smallpox

vaccine, which is supposed to be one of the strongest, you still continue to give that vaccine every so many years. Tetanus and diphtheria, you boost those. If you prevent whooping cough in the younger age group where it's always occurred, do you think you're going to prevent it forever just with immunizing the child in five years?

DR. CULBERTSON: Dr. Murray, I'd like to ask another question.

DR. MURRAY: Dr. Culbertson.

DR. CULBERTSON: What does anyone know about the relation of dose? Is it axiomatic that the more you give the more immunity you get? We had one which showed the reverse, one series of our experiments. If we gave too much, we didn't get as much.

I suppose there's a minimum, but I think maybe there is a possibility one might give too much, and I think this is something that ought to be carefully looked at.

DR. MURRAY: Dr. Perkins.

DR. PERKINS: One factor that we are investigating in England -- I'd like to know if anyone else is doing that over here -- is Dr. Preston whose work was mentioned this morning has prepared now monospecific antisera of types I to IV and finds that the early isolated strains in the 1945 to 1955 period were all I, III strains, and these are the strains from which we were making our

vaccines. But now, using the I, III strain vaccines, in the face of these epidemics now, he's only isolating II, IV strains, and, of course, it's a long shot in the dark to say whether, in fact, the I, III immunity that one is getting would give one protection against a II, IV strain that happens to be going around now or whether the vaccination indeed has caused this change in the strain, antigenic variant.

Whether anyone has got evidence in this country would be very interesting to know.

DR. KENDRICK: Dr. Eldering has some information on that.

DR. ELDERING: I'm talking too much.

DR. MURRAY: Well, it might be very interesting to speculate about the reasons for failure, but I think we should focus this afternoon in this particular session on matters of toxicity.

Dr. Dettwiler.

DR. DETTWILER: Dr. Perkins was good enough to tell us of the situation in Britain, and I believe we have representatives from Netherlands and Canada, of course, and Denmark. I'd like to know whether they use toxicity as a criterion and what do they think of a test of this sort?

DR. MURRAY: Do you care to talk to that, Dr. Cohen?

DR. COHEN: Well, I think we're more or less in the position of the United States manufacturers now. We were very lucky to escape, because the mouse toxicity test wasn't accepted into Holland until recently, the difficult situation of many American producers.

We recently published our material in the Dutch paper, and when you read it you will see we couldn't meet with USA mouse toxicity test during the years.

We have discovered now that it might be that PPLO, with which our mice were heavily infected, may play a role here. Now we're importing mice from the States directly, and we're getting acceptable toxicity tests, getting weight gains of about 4 to 5 to 6 grams. We're very lucky we meet the U. S. toxicity test at this moment.

DR. MURRAY: Would you care to comment, Dr. Wilson?

DR. WILSON: I think Dr. Corkill, Mr. Chairman, has had the most experience in this area.

DR. J. M. CORKILL (Connaught, Toronto): In testing our products for toxicity we have followed your later outlines in testing mice. But some years ago I was impressed that when we were having a certain number of severe reactions in children we had noticed that guinea pigs injected with about five times the human dose-- We were killing pigs and producing in them certain typical (Putnam) pictures of pathology. So I have always maintained

this guinea pig injection in our production lots.

As a result of that I have at times found that lots which have passed the mouse tests very well have killed guinea pigs and have produced perhaps some of these pictures of ulcers.

It's sort of like a mirror-like hemorrhage produced in a very severe lot or in a very toxic lot. These mirror hemorrhages may occur in the walls of the stomach. They certainly occur in the walls of the large intestine, and you will find that the small intestine is very inflamed and red, and in some very toxic lots you may find this swelling in the duodenal loop.

DR. MURRAY: So you are using a guinea pig test as a test for toxicity?

DR. CORKILL: Yes. By the way, this injection of five times the human dose must be given intraperitoneally. If it's just given subcutaneously there is no death of the pig or no change.

DR. WILSON: We also use the NIH test, Mr. Chairman, but this is additionally.

DR. MURRAY: From the manufacturing point of view, your guinea pig test seems to be more hazardous.

DR. WILSON: We are fortunate in the strain of mice I think, Mr. Chairman.

DR. CORKILL: We have no clinical trials to prove

really that would pass potency tests.

DR. MURRAY: Dr. Spaun, would you care to comment on the Danish situation?

DR. J. SPAUN (Copenhagen, Denmark, Visiting Scientist): We do these traditional toxicity tests on a similar line as you do here but we don't know what they tell us.

DR. CULBERTSON: I wish to ask what the time relations on the guinea pig deaths were. How long did it take them to die after you gave the material?

DR. CORKILL: Some can die within 24 hours.

DR. CULBERTSON: How long do you let them go when you do your tests?

DR. CORKILL: We follow them along for two weeks but the deaths would all occur within 48 hours.

DR. CULBERTSON: It's like the mice maybe.

DR. PERKINS: Wouldn't that guinea pig test be the same as your proposed test of acute toxicity, Dr. Culbertson?

DR. CULBERTSON: That's what I thought from what he said. Perhaps it was the same sort of a situation.

DR. PERKINS: Because you're obviously getting lesions in the intestines very early from this test that they are doing there.

DR. CULBERTSON: Our knowledge of that early

period in the mice is in its infancy. We haven't studied it enough. In the last two weeks we just begin to see this hemorrhage early.

DR. MURRAY: May I ask, Dr. Culbertson, would you think that it is profitable to pursue further work with germfree mice or perhaps specific-pathogen-free mice or --

DR. CULBERTSON: Well, germfree mice certainly offer a medium for study. There are many difficulties in doing germfree mice. I showed you two strains or one strain of our mice had apparently two different viruses in it and they failed to gain, even the normal saline controls, when they had this.

And the variations in them may be as great as the ones outside, but different types of variations.

You see, you have the problem of diet there. You have got to put enough vitamins in that stand autoclaving and all this sort of thing that creates a terrific problem from the standpoint of a routine test at the moment.

It would seem to me like if you would inject a mouse with ascending amounts of this or descending amounts and cut the test off before this colonic thing has a chance to cause a mortality that you might-- And we haven't done enough of this to know whether it will work or not,

but it might be a test for acute toxicity just as you test a chemical, and you don't wait for them to die in seven days. You test them and you get your death point within a very short time.

And that is what put this idea in our heads of trying these large doses.

And don't misunderstand me. I just say this is a possible way to look at this, to give these folks who sweat over these tests some way they can get results they can depend upon.

I think this is the problem they have repeatedly. They think they have got a vaccine prepared, and then they can't decide whether it's good or bad. And what we need is something that would give them an answer on it.

DR. MURRAY: Dr. Munoz.

DR. MUNOZ: In respect to these toxicity tests, I think nobody has made it absolutely clear what they are measuring. Are they measuring the heat-labile toxin or the heat-stable toxin? And I think an effort should be made in any studies that you do in the future to differentiate between these two toxins, because the heat-labile toxin is extremely potent, especially if the cells happen to (~~lise~~ lyze), like when you use phemerol, for example, as a preservative. So this should be kept in mind.

DR. CULBERTSON: I think that is a good idea.

DR. MURRAY: Dr. Dettwiler.

DR. DETTWILER: Earlier this afternoon I believe Dr. Pittman mentioned that Dr. Bell had some clinical studies under way trying to show the correlation between mouse toxicity and humans. Can you tell us more about those studies, and are they continuing?

DR. PITTMAN: The first data that we received from Dr. Bell looked quite encouraging. There was a difference -- it was not statistically significant -- but with one vaccine in the mouse which gave no dose response curve, the percentage of febrile reactions over 101.6 was 12 per cent.

With another vaccine, which gave a dose response curve in toxicity, it was 19 per cent.

We thought this looked very nice. But he told me -- I do not have the data yet -- that eventually they came out practically the same. These were done in a group of children that have quite a number of types of infections. We thought at first they looked very pretty, but the latest report was that it did not show the difference.

DR. WHALEN: I think it's very significant what Dr. Munoz has said about the toxin you're measuring.

You said, Dr. Pittman, you had 3 per cent deaths with the Dettwiler preparation, and yet the other workers had a higher percentage.

Now, this probably had the heat-labile toxin in it from the way that it was prepared.

We have a lot of material here that you tested six times and it did not pass. We tested it seven times and it passed each time. This was probably heat-stable toxin and it may be your mice were more sensitive to the heat-stable toxin than were our mice.

DR. MURRAY: Well, there doesn't seem to be any easy solution to this.

Any other comments? Discussion?

(No response.)

If not, then we'll adjourn and we'll reconvene tomorrow morning, same place, at 9:00 a.m.

(Whereupon, at 4:45 p.m., the meeting was adjourned, to be reconvened at 9:00 a.m., Tuesday, October 22, 1963.)

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